

evolution

2017

BOTANY - I: EMBRYOLOGY

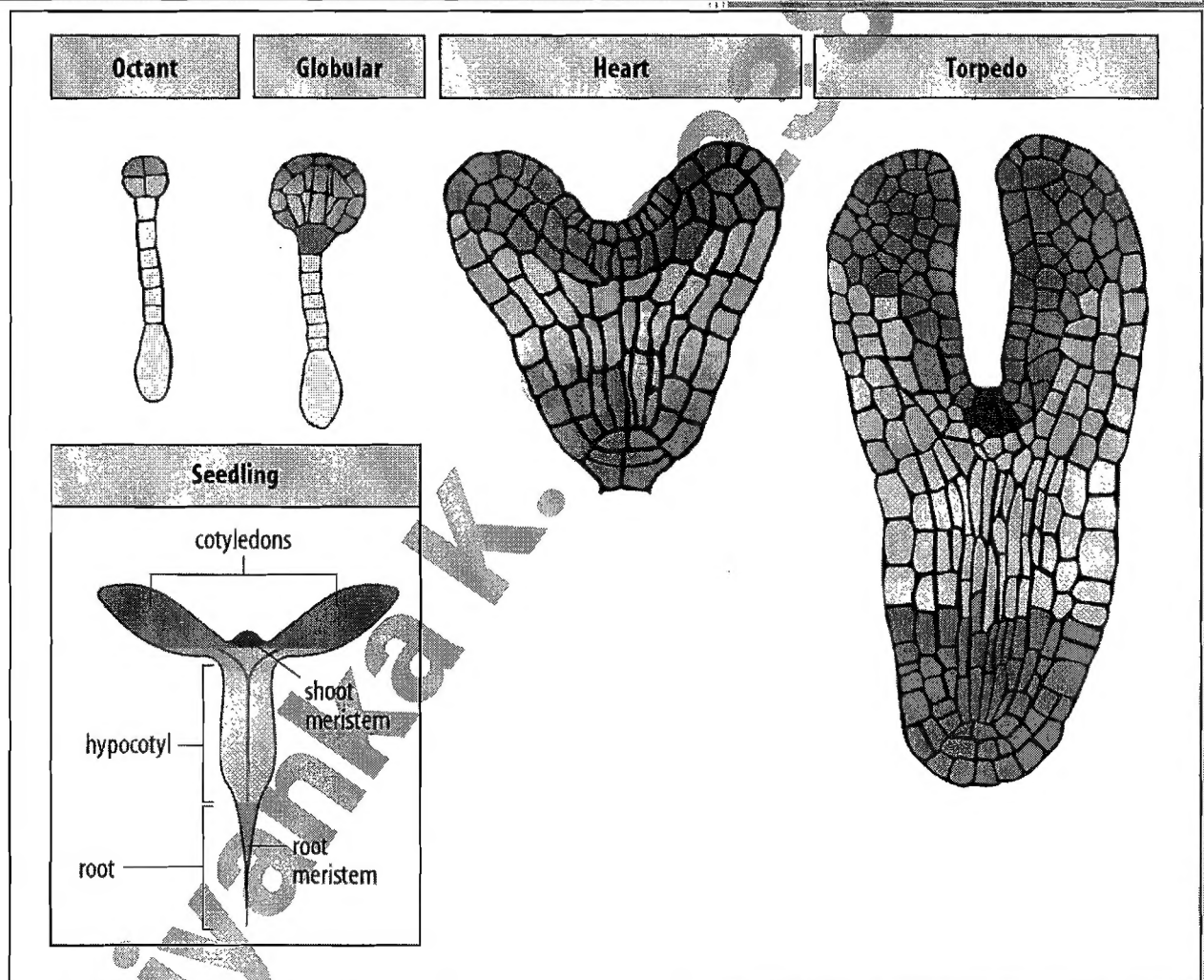


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0. Prescribed syllabus

- Development of male and female gametophytes
- Pollination
- Fertilization
- Endosperm — Its development and function
- Patterns of embryo development
- Polyembryony
- Apomixis
- Applications of palynology
- Experimental embryology including pollen storage and test-tube fertilization

1. Angiosperm life cycle

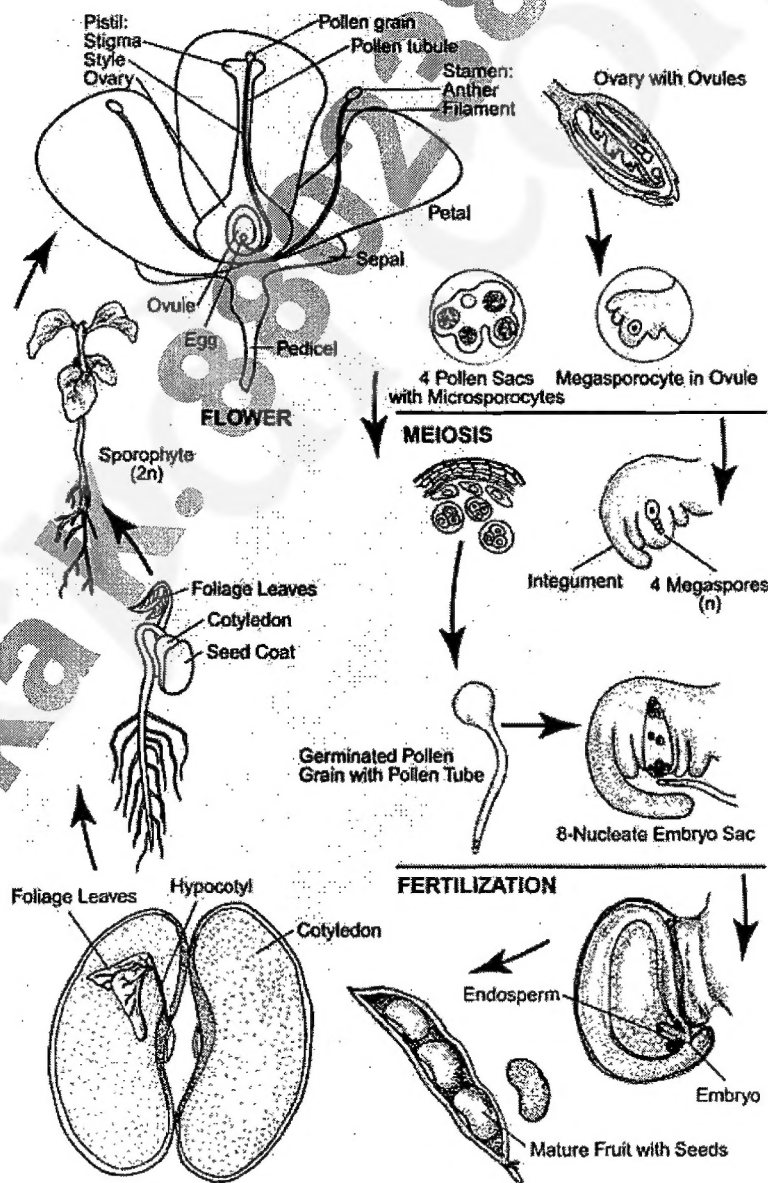
An overview

Like other plants, the angiosperms alternate a sporophytic generation with a gametophytic one, a **sporic meiosis**. Angiosperm **sporophytes** produce through **meiosis** (reduction division) **two kinds of spores** in specialized structures of their flowers, **microspores in the anthers** and **megaspores in the ovules** contained within the ovaries.

The **gametophytes**, which develop from the spores, are much reduced in size. The **microgametophyte** consists of only three cells and is the germinated pollen grain. The **megagametophyte** is the mature embryo sac, a seven-celled structure in the ovule surrounded by, and dependent upon, sporophyte tissue.

Development of gametophytes

Haploid **microspores** develop from **microsporocytes** in the anthers and give rise to pollen grains containing two cells: the **tube cell** and the **generative cell**. At about the time of pollination, the latter cell divides and produces two **sperm**. This three-celled pollen grain is the immature male gametophyte (**microgametophyte**).



The female gametophyte, the **megagametophyte**, develops in the ovary at the same time the male gametophyte is developing in the anthers. While the process is variable among taxa, about three-quarters of the flowering plants go through the following steps.

One **megasporocyte** is contained in each of the young ovules within the ovaries in the flower buds. The ovule is attached by a stalk, the **funiculus**, to the **placenta** on the ovary wall and, at this stage, is essentially a lump of tissue, the **nucellus**, covered by two tissue layers, the **integuments** (which wrap almost completely over the ovule but leave a small opening, the **micropyle**, at one end). Three of the haploid megaspores produced by the megasporocyte disintegrate almost immediately and the remaining one divides by three successive mitotic divisions to produce eight nuclei in an **embryo sac** within the elongated, swollen megaspore. The nuclei cluster, first in groups of four at either end of the sac and then one nucleus from each end, migrates to the center. The two migrating nuclei are called the **polar nuclei** and they form a **polar cell** when walls develop around them. Cell walls also form around the three nuclei left at the end of the cell opposite the micropyle, the **chalazal end**. The chalazal end cells are the **antipodals**. At the micropylar end, the three nuclei are organized into the **egg apparatus** and walls form around each of them also. One cell is the **egg cell**, the two others are **synergids** (helpers). All three look alike, but only the egg continues to develop; the synergids deteriorate as do the antipodals on the opposite end of the sac. The **embryo sac** at this stage is the female gametophyte (megagametophyte). Before further development can occur and seeds are produced in the ovary, two events must occur: pollination followed by fertilization.

Pollination

Pollination is the mechanical transfer of pollen grains from an anther to a **stigma**, the receptive end of a carpel. Pollination is accomplished by a variety of physical dispersal agents such as wind, water, and gravity or many kinds of animals including insects, bats, birds, and small rodents. The variations in floral structure are, in large part, adaptations to achieve pollination success. Most pollination is between flowers located on separate plants (**cross-pollination**), but in some taxa **self-pollination** occurs when pollen from the anthers falls on stigmas of the same plant.

Fertilization

If the pollen grain lands on the stigma of a genetically compatible flower, it absorbs moisture and a **pollen tube** emerges through a pore in the wall. The germinated pollen grain with its pollen tube and three nuclei is the **mature male gametophyte**. The tube grows downward toward the ovary through special tissues in the style, penetrates the embryo sac, usually through the micropyle (destroying a synergid in the process), and discharges its contents. The tube nucleus disintegrates while one of the sperm nuclei

fuses with the egg nucleus, forming a **zygote**. The other sperm fuses with the polar cell, forming the **endosperm nucleus**. In other words, a **double fertilization** occurs: Both sperms fuse with embryo sac nuclei. Double fertilization is a characteristic of the angiosperms and results in a **polyploid** endosperm tissue. (Polyploidy refers to the number of sets of chromosomes the cell contains; plants with more than the diploid two sets are polyploids. The endosperm tissue may be *triploid* [$3n$] or more depending upon the species.)

If no pollen tube and its contents reach an ovule in the ovary, the ovule aborts with no further development. Lacking chemical signals (hormones) from a developing seed, the ovary, too, may wither and die. If double fertilization does occur, the ovule develops into a **seed** and the entire ovary into a **fruit**.

2. Development of male gametophyte

Microsporogenesis

The Construction of Microsporangium

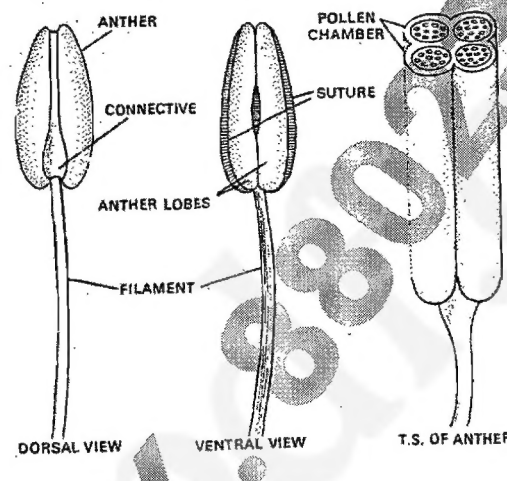


FIGURE 1: THE CONSTRUCTION OF STAMEN

The stamen (Fig. 1) is the male reproductive organ of the flower. It produces the male spores or the pollens within the pollen chambers of the anther. The pollens are dispersed by various means to the stigma of a compatible flower, where it germinates to give rise to the male gametophyte (the pollen tube). A pollen tube contains two male gametes, both of which participate in the double fertilization.

The stamen has a structure consisting of two parts — a lower sterile **filament** and an upper fertile **anther**. The **connective** in most angiosperms connects the filament with the anther. The **anther** is typically **two-lobed** with **each anther lobe having a pair of pollen sacs or microsporangia**. Thus, characteristically each anther has four microsporangia. At maturity, the two sporangia in a lobe become confluent because of degradation of partition between them. More than four microsporangia in an anther are extremely rare but less than four is frequent and characteristic of many plants, for example in *Moringa* [2 sporangia], *Wolffia* [2 sporangia], *Hibiscus rosa-sinensis* [1 sporangium], *Arceuthobium* [1 sporangium], and *Abelmoschus* [1 sporangium].

Male sexual development

In most of the angiosperms, the male sexual development begins somewhat before the female sexual development. The female sexual differentiation begins when almost 50% of male differentiation is accomplished (Dalwitsch, 2001). The following are the principal stages of male sexual development in angiosperms (Fig. 2).

1. The young anther is a homogeneous mass of meristematic cells surrounded by an epidermis. The process of reproductive differentiation begins when groups of hypodermal cells in each of the four corners become distinguished from the surrounding cells by their larger size, dense cytoplasm and prominent nuclei. These cells are arranged in single vertical rows of cells [Malvaceae and Asteraceae] or plate-like or crescent-shaped vertical rows [in some members of Lamiaceae such as *Mentha*]. They form the **archesporium** of the anther. The number of archesporial cells varies in different taxa. Sometimes, as in *Doryanthes* and *Holoptelea*, the archesporium is not differentiated in the hypodermis, but develops from more centrally situated cells.
2. The archesporial cells divide periclinally resulting into outer **primary parietal cells** and inner **primary sporogenous cells**.
3. The primary parietal cells undergo repeated periclinal and anticlinal divisions giving rise to 3-5 concentric layers, which eventually form the anther wall. The anticlinal divisions also take place to increase the surface area, while the periclinal divisions add layers.
4. The primary sporogenous cells generally undergo a few mitotic divisions. The subsequent generations of sporogenous cells are regarded as Secondary Sporogenous cells.
5. The divisions in the sporogenous cells and the parietal cells result in the increased volume of the anther. The epidermis of the anther goes through rapid anticlinal divisions to cope with the expansion in size.
6. The sporogenous cells usually separate prior to going for the sporogenic meiosis. The

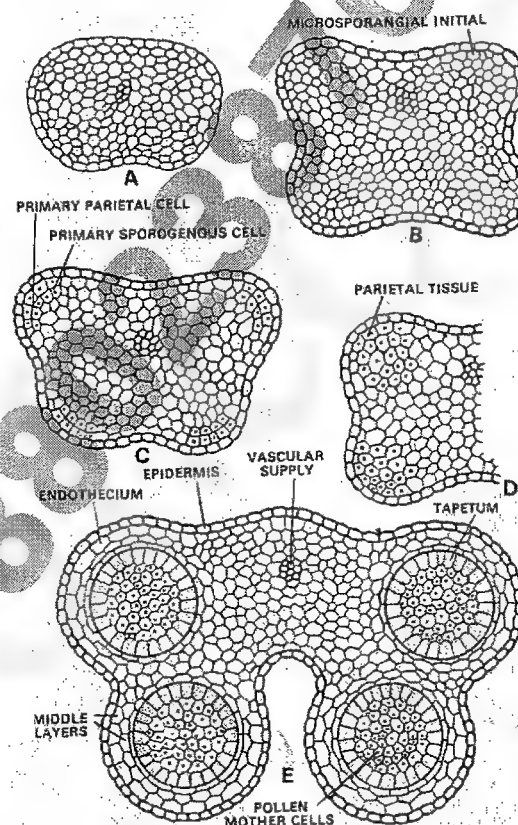


FIGURE 2: DEVELOPMENTS TOWARDS POLLEN MOTHER CELLS

separated sporogenic cells are regarded as **microspore mother cells**.

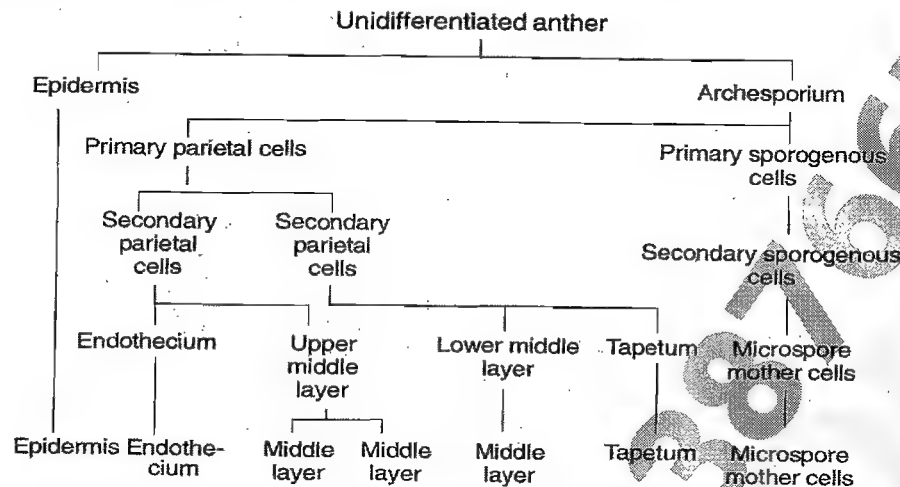


FIGURE 3: ONTOGENY OF ANTHER LAYERS

Histology of the mature anther wall

The mature anther wall is made of **four layers**.

1. Epidermis
2. Endothecium
3. Middle layers
4. Tapetum

EPIDERMIS

This is the outermost wall layer of the anther made up of tangentially stretched and flattened cells. In xerophytic plants, the epidermal cells are stretched to such an extent that they lose contact among themselves and appear as withering remains in a mature anther. Stomata may occur in the epidermis as in *Alangium*.

ENDOTHECIUM

This is the subepidermal layer of the anther wall. Mostly there is a single layer of endothecium, but sometimes, as in *Coccinia indica*, it is two layered. The cells of endothecium are radially elongated and attain their maximum development when pollen grains mature. The radial and inner tangential walls of these cells have characteristic fibrous thickening bands. In some cleistogamous species where flowers do not open and in the members of Hydrocharitaceae fibrous bands are altogether absent in the endothecium. The presence of fibrous bands, differential expansion of inner and outer tangential walls and hygroscopic nature of endothelial cells play an important role in the dehiscence of anthers.

MIDDLE LAYERS

One to three layers, lying next to endothecium, are the middle layers. The cells of these layers are ephemeral and degenerate almost completely before the pollen mother cells undergo meiosis.

TAPETUM

Tapetum, the innermost wall layer of the anther, usually occurs as a single layer around the sporogenous tissue. Physiologically it is extremely crucial for normal pollen development. Tapetal malfunctioning is one of the frequent causes behind male sterility in Angiosperms.

The cells of the tapetum are relatively large and centripetally extended. They have dense cytoplasm and prominent nuclei. Sometimes the cytoplasm of tapetal cells is pigmented; for instance, red-brown pigments are present in apple and violet pigments in *Anemone*.

Tapetum is present in the form of a homogeneous layer, which surrounds the sporogenous tissue. In some taxa, however, a **dimorphic tapetum** is present. For example, in *Alectra thomsonii* and *Nigella* the tapetum on the protuberant side of the anther, formed from the derivatives of primary parietal cells, is called **P-tapetum**; and on the connective side, where it differentiates from the cells of the connective and/or septum between the two pollen sacs, is referred to as **C-tapetum**. The cells of C-tapetum are relatively large than those of P-tapetum.

As the sporogenous cells undergo meiosis, the nuclei of tapetal cells also divide and the cells of mature tapetum become usually multinucleate. Sometimes, polyploidy and polyteny can also be seen. The unusual nuclear constitution of the tapetum is because the tapetal nucleus can divide by any of the following methods.

- (a) **By endomitosis.** In such divisions, the nucleolus and nuclear membrane remain intact and chromosomes split longitudinally. It leads to polyploidy. Examples - *Spinacia oleracea*, *Cucurbita pepo*.
- (b) **By normal mitosis but no cytokinesis.** It leads to multinucleate condition. Example - *Zea mays*.
- (c) **Formation of restitution nuclei.** In *Lilium canadense* and few other species, the nuclear division is almost normal till anaphase. Subsequently, the chromosomes fail to separate and as such a dumbbell-shaped tetraploid nucleus is formed. This nucleus is known as restitution nucleus.
- (d) **By endoreduplication.** The chromosomes keep replicating themselves but do not split longitudinally. It results into polyteny.

TYPES OF TAPETUM. The following two types of tapetum have been recognised in angiosperms:

1. **Amoeboid or plasmodial tapetum.** In this type, the radial and inner walls of the tapetal cells break down early due to the action of hydrolytic enzymes. The protoplasm of these cells enters into the anther locule and fuses to form a common mass called **tapetal periplasmodium**. This mass surrounds the pollen grains until they are mature and provides them proper nutrition. When pollen grains mature, the periplasmodium also contributes to their exine by providing sporopollenin precursors. Electron microscopic studies have shown that periplasmodium is a highly organised functional structure. The nuclei present in the periplasmodium also divide. The amoeboid tapetum is considered to be of primitive type and is found in *Arum*, *Alisma*, *Typha*, *Tradescantia*, *Sagittaria*, *Potamogeton*, etc.
2. **Secretory or glandular tapetum.** The cells of the secretory tapetum remain attached to the middle layer until the development of pollen grains. At the pre-meiotic stage of the pollen mother cells, tapetal cells are very thin and possess almost all cell organelles like mitochondria, plastids, dictyosomes, etc. Some spherical structures, called **pro-ubisch bodies**, are also present in this cell. Just before the pollen mother cells undergo meiosis, the walls of the tapetal cells become thick and there is considerable increase in the number of ribosomes and pro-ubisch bodies. With the completion of pollen development pro-ubisch bodies pass, into the anther locule from the tapetal cells and these are now called **ubisch bodies**. They perhaps help in the formation of exine of pollen grains. The secretory tapetum is more advanced and found in most of the angiosperms.

Functions of tapetum. Tapetum is very critically associated with the development of pollen grains. If in an anther tapetum degenerates before microsporogenesis, the pollen grains produced are sterile or abortive. Tapetum also helps in the transport of food material to the inside of the anther. The food material stored in tapetal cells is not utilised in the early stages of anther development. However, after pollen mother cells have undergone meiosis the protoplast of the tapetal cells enters into the pollen chamber to form periplasmodium which provides nutrition to the developing pollen grains and also helps in the formation of exine. The tapetum also contributes material for the pollen wall synthesis (**ubisch bodies**).

In insect pollinated species, the surface of the pollen grains is covered by an oily layer called **pollenkitt**. The sticky nature, colour and smell of pollen grains is due to its presence. The substances necessary for the synthesis of pollenkitt are secreted by the tapetal cells.

Microsporogenesis process

Microspores (pollen grains) develop from the sporogenous tissue. The primary sporogenous cell may directly function as microspore mother cell or they undergo several mitotic divisions to form microspore mother cells (as shown in Fig. 3). All sporogenous

cells of an anther are capable of forming microspores but usually some of these cells degenerate and provide nourishment to the active sporogenous cells. The surviving cells are called Microspore or Pollen Mother Cells (PMCs)

Each PMC undergoes meiosis and forms four haploid microspores. The process of formation of microspores from sporogenous tissue is known as **microsporogenesis**.

Microspores are formed by reduction division of microspore mother cells and this division is completed in two steps. In the first step, known as **meiosis-I**, two haploid cells are formed, and the second step, called **meiosis-II**, is a normal mitotic division. It is believed that the stimulus which prompts sporogenous cells to undergo meiosis originates in the vegetative shoot apex. This stimulus affects only sporogenous cells and not any other cell of the anther. As all microspore mother cells of an anther locule are interconnected by plasmodesmata, their meiotic stages are closely synchronised.

In **meiosis-I**, the homologous chromosomes (chromosomes derived from maternal and paternal gamete nuclei) of the microspore mother cell come together and pair up forming bivalents, **Prophase-I** is characterized by genetic crossing over. At **metaphase I** the bivalents are arranged across the equatorial plate. Subsequently the homologous chromosomes of each bivalent are gently pulled to two opposite poles by spindle fibres. Thus by the end of meiosis I chromosomes are separated into two haploid sets. In **meiosis-II** these two haploid sets of chromosomes divide mitotically, resulting in the formation of four haploid nuclei. Each of these nuclei eventually forms a pollen grain.

Inner to the original cell wall, a special callose wall is formed around each microspore mother cell during early meiosis. It increases in thickness during meiosis, reaching at its maximum at tetrad stage. As tetrads mature the special wall dissolves.

The microsporogenesis in Cyperaceae is different from other families. Here, out of the four haploid nuclei formed by the reduction division of microspore mother cell, only one is functional and the remaining three degenerate. Thus in Cyperaceae each microspore mother cell forms only a single microspore.

Cytokinesis

During meiotic division of microspore mother cell, the wall formation may be successive or simultaneous type.

[I] Successive type

In this type of wall formation, which is found mostly in the monocotyledons, a cell plate is laid down between two daughter nuclei after **meiosis-I**. This wall gradually extends towards the periphery on both sides dividing the cell into two equal halves. Each cell of the dyad thus formed

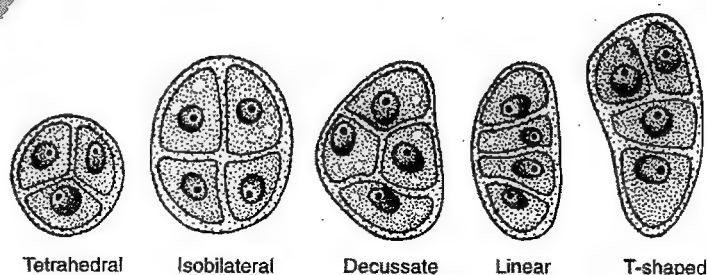


FIGURE 4: THE FIVE TYPES OF POLLEN TETRADS IN ANGIOSPERMS

undergoes **meiosis-II**. The wall formation between the two daughter nuclei occurs in the same way as after meiosis-I. This results in the formation of a microspore tetrad. The second meiotic division may not be synchronous in both the cells of the dyad.

[II] Simultaneous type

Simultaneous type of cytokinesis is common in the dicotyledons. In this type, there is no wall formation between the two daughter nuclei after meiosis-I and as such instead of a dyad, simply a binucleate cell is formed after meiosis-I. The two daughter nuclei then undergo second meiotic division, which is usually synchronous. At this stage, four daughter (haploid) nuclei lie in the common cytoplasm. The wall formation begins in the form of four peripheral constrictions, which gradually extend from periphery towards the centre. These constrictions eventually meet at the centre of the cell and divide the cytoplasm into four haploid microspores.

Microspore Tetrads

The microspores of a tetrad are separated from each other by callose wall. There is also no connection between the microspores of different tetrads in the anther locule. However, in some orchids microspores do show cytoplasmic connections.

The four microspores formed from a microspore mother cell are usually arranged in tetrahedral or isobilateral tetrads. In **Tetrahedral tetrads**, which are formed as a result of simultaneous division, the four microspores lie at the four corners of a tetrahedron. When seen from an angle, only three microspores are visible and the fourth lies at the back. In **Isobilateral tetrads**, which are formed by successive divisions, the four microspores are arranged in one plane.

Besides the above two types, sometimes microspores may be arranged in **Decussate** (e.g., *Magnolia*, *Crocus*, *Atriplex*), **T-shaped** (e.g., *Aristolochia*, *Butomopsis*) or **Linear** (e.g., *Halophila*) tetrads.

In the species *Aristolochia elegans*, all the five types of pollen tetrads are seen.

The microspores of a tetrad usually separate from each other as the anther matures. However, sometimes (e.g., *Drimys*, *Annona*, *Acacia*, *Drosera*, *Typha*, *Elodea*, etc.) they do not separate even at maturity and even the tetrads are stuck together in groups which may contain as many as **64 pollen grains**. Such groups are called **compound pollen grains**. In Asclepiadaceae all pollens in a pollen sac are united in a single compact mass, known as **Pollinia**. The pollinia are also formed in Orchidaceae but in certain members of this family the pollinium is less compact as it comprises smaller groups of pollen grains. Such loose groups are termed as **massulae**. The pollen grains of a massulae are loosely joined among themselves by means of a **viscin** thread. In Ericaceae and Empetraceae the pollen grains are permanently united in tetrahedrons.

(Viscin is a clear, viscous, tasteless and sticky substance extracted from the mucilaginous sap of the mistletoe (*Viscum album*) and several other plants. The major

component of viscin include neutral sugars like xylose and arabinose, amino acids, uronic acids and proteins. Presence of Viscin in orchids is one of the characteristic morphological features. The pollen grains are usually bound together by threads of a clear, sticky substance called viscin in masses called pollinia. Two basic kinds of pollinia exist: one has soft, mealy packets bound together to a viscin core by viscin threads and is called sectile; the other kind ranges from soft, mealy pollinia, through more compact and hard waxlike pollinia masses).

Fate of the microspores

The callose wall of the tetrad stage is broken down by an enzyme called callase and the freed pollen grains grow in size and develop their characteristic shape. Then the microspores undergo differentiation and maturation. At the structural level, they form a resistant outer wall called the exine and an inner wall called the intine. The exine is made up of a resistant compound called sporopollenin; the intine is made up of cellulose and pectin. The exine often bears spines or warts, or is variously sculptured, and the character of the markings is often of value for identifying genus, species, or even cultivar. Except in the case of some submerged aquatic plants, the mature pollen-grain always has a double wall.

In most flowering plants, germination of the microspore begins before it leaves the microsporangium. At maturity, each pollen grain contains a **vegetative** (non-reproductive) cell and a **generative** (reproductive) cell containing two nuclei: a tube nucleus (that produces the pollen tube) and a generative nucleus (that divides to form the two sperm cells).

Mature pollens are then transferred by various modes (depending on the species) to the compatible stigma. The transfer of pollen grains to the female reproductive structure (**pistil** in angiosperms) is called **pollination**.

Male gametophyte

The normal course of male gametophyte development

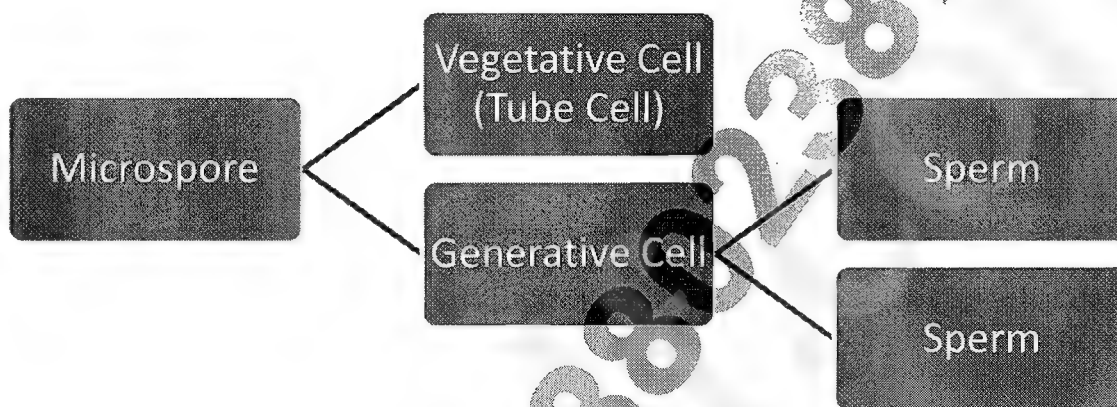
The microspore is the first cell of male gametophyte. The germination of microspore starts *in situ*.

Microspore divides by asymmetric mitotic division into a large tube cell and small generative cell. The tube cell is also called vegetative cell. The smaller generative cell is completely enclosed within the cytoplasm of the larger vegetative cell. Pollination takes place at this two celled stage in about 70% of angiosperms. In such examples, the further development of the male gametophyte takes place upon stigma.

For further development, a mitotic division of the generative cell generates two sperm cells which remain connected by a common extracellular matrix with potential intercellular connections.

In about 30% angiosperms, pollination occurs at three celled stage. This means, the generative cell divides into two sperms before the release of the pollen grains. It is well studied in families like Brassicaceae and Asteraceae.

Thus, the basic pattern of male gametophyte development in angiosperms is as follows.

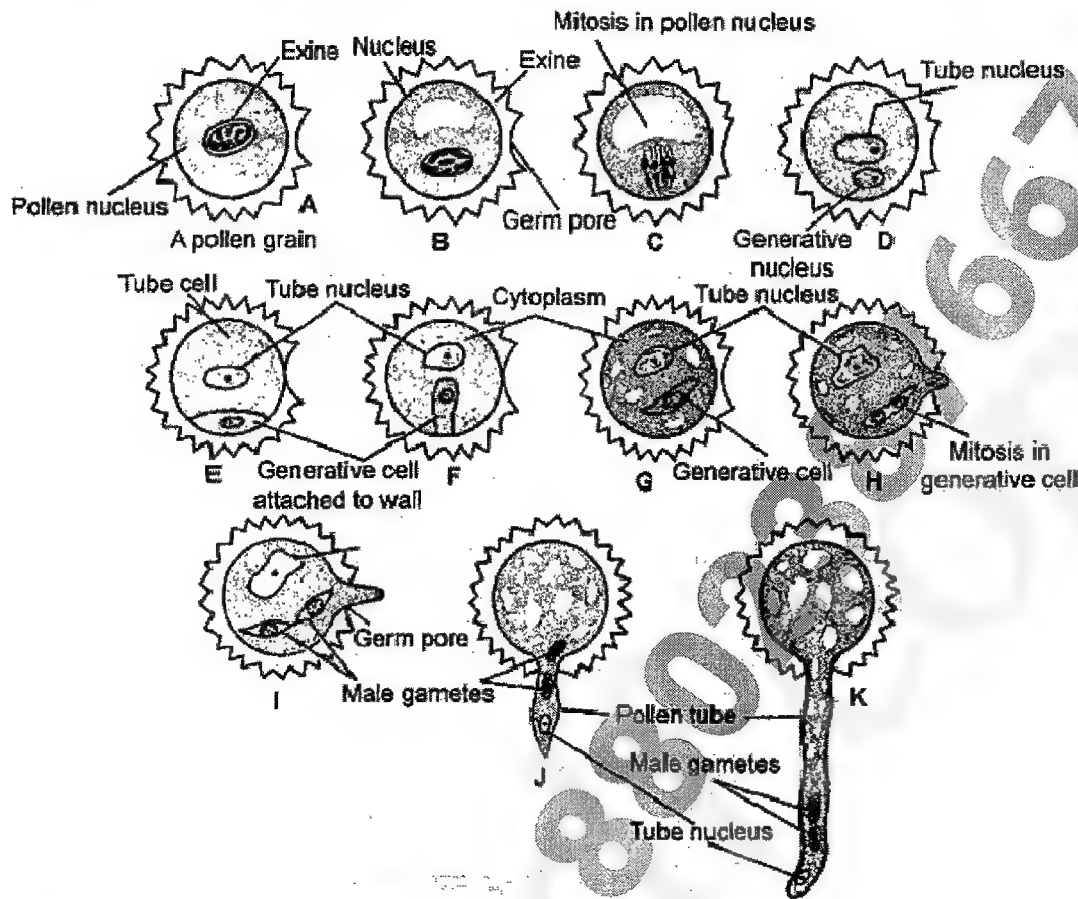


The process of male gametophyte formation

Pollen grain expands by absorbing the liquid from the moist surface of stigma. Stigma provides boron, sugar amino acids etc. The intine comes out in the form of pollen tube, from germ pores: Growth of pollen tube is apical and chemotropic. The pollen tube was first observed by G.B. Amici (1824) in *Portulaca*.

The pollen grains are either monosiphonous (with one pollen tube) or polysiphonous (with more than one pollen tubes) e.g., members of Cucurbitaceae and Malvaceae.

The generative nucleus divides mitotically to form two male gametes called sperm. The male gametes are non-motile and amoeboid. They are slightly unequal in size. The larger sperm has several mitochondria and chloroplasts. The smaller sperm cell has a limited number of mitochondria and usually no chloroplast.



Male germ unit (MGU)

In flowering plants, the vegetative nucleus and the two sperm cells are proposed to form a functional assemblage, the male germ unit (MGU). The assemblage was first reported by Russel and Cass in 1981 in *Plumbago zeylanica*. Later Dumas (1984, 1985) coined the term MGU.. Thus, the shared extracellular matrix of the two sperm cells and the physical association of one sperm cell to the vegetative cell nucleus forms a linkage of all the genetic material in the pollen grain, termed the male germ unit.

In 2002, Lalanne and Twell identified *mud* and *gum* genes in *Arabidopsis*, which are essential for formation and integrity of the MGU.

McCue, Cresti *et al.* (2011) discovered that one sperm cell has a cytoplasmic projection in contact with the vegetative cell nucleus. This arrangement is found in both the monocot and dicots. The cytoplasmic projection is formed by microtubule elongation shortly after the formation of the generative cell.

The cytoplasmic projection potentially plays other important roles also. They facilitate communication between the somatic vegetative cell nucleus and the germinal sperm cells, via RNA and/or protein transport.

Pollen Embryo Sacs (Nemec Phenomenon)

Generally the developing male gametophytes are either 2 or 3 nucleated and spherical in form. But Nemec (1898) observed eight nucleated embryosac like male gametophytes in petaloid anthers of *Hyacinthus orientalis*. Such abnormal male gametophytes are called as pollen embryosacs.

The microspore here increases in size to form a sac like structure. The nucleus undergoes three mitotic divisions to form 8 nuclei. Of these, 3 lie at the end where exine is still intact, 3 at the opposite end and 2 in the middle.

The developmental basis:

There are different opinions in this regard.

According to Nemec, this condition arose as the result of:

- disintegration of generative nucleus, and
- three successive divisions of vegetative nucleus.

On the other hand, de Mole (1923) stated that the abnormality arose due to the division of generative nucleus rather than vegetative nucleus.

Stow (1930, 1934) studied the development and behaviour of embryosac like pollen grains by conducting series of experiments. He observed large number of disintegrating pollen grains along with few embryo sac like pollen grains (gametophytes) in some anthers. According to him the disintegrated pollen grains secrete, "necro-hormones" into anther locule. Due to this, the surviving pollen grains increase in their size. The pollen nucleus undergoes three successive division to form eight nuclei. All the eight nuclei are organised into embryosac like structure. He also observed pollen embryosac behaviour by placing them on agar plates along with some normal pollen grains of another variety. He noticed penetrating pollen tube of normal pollen grain in to pollen embryosac.

Finally, he concluded that, under normal conditions male potency is dominant and that lead to the formation of vegetative cell and generative cell. Whereas under abnormal conditions in presence of nechrohormones the female potency is dominant over male potency and lead to the formation of embryosac like male gametophytes.

3. Ovules

General Introduction to Ovule

An ovule is a structure found in seed plants that develops into a seed after fertilization. In angiosperms, or flowering plants, the ovule is found within an ovary which becomes fruit. In conifers and other gymnosperms, the ovules are borne on the surface of an ovuliferous (ovule-bearing) scale, usually within a cone, and are not enclosed by ovary. Prior to fertilization, the ovule consists of the female gametophyte, the nucellus, and integuments, which in angiosperms are attached to the placental wall of the ovary through a structure known as the **funiculus**.

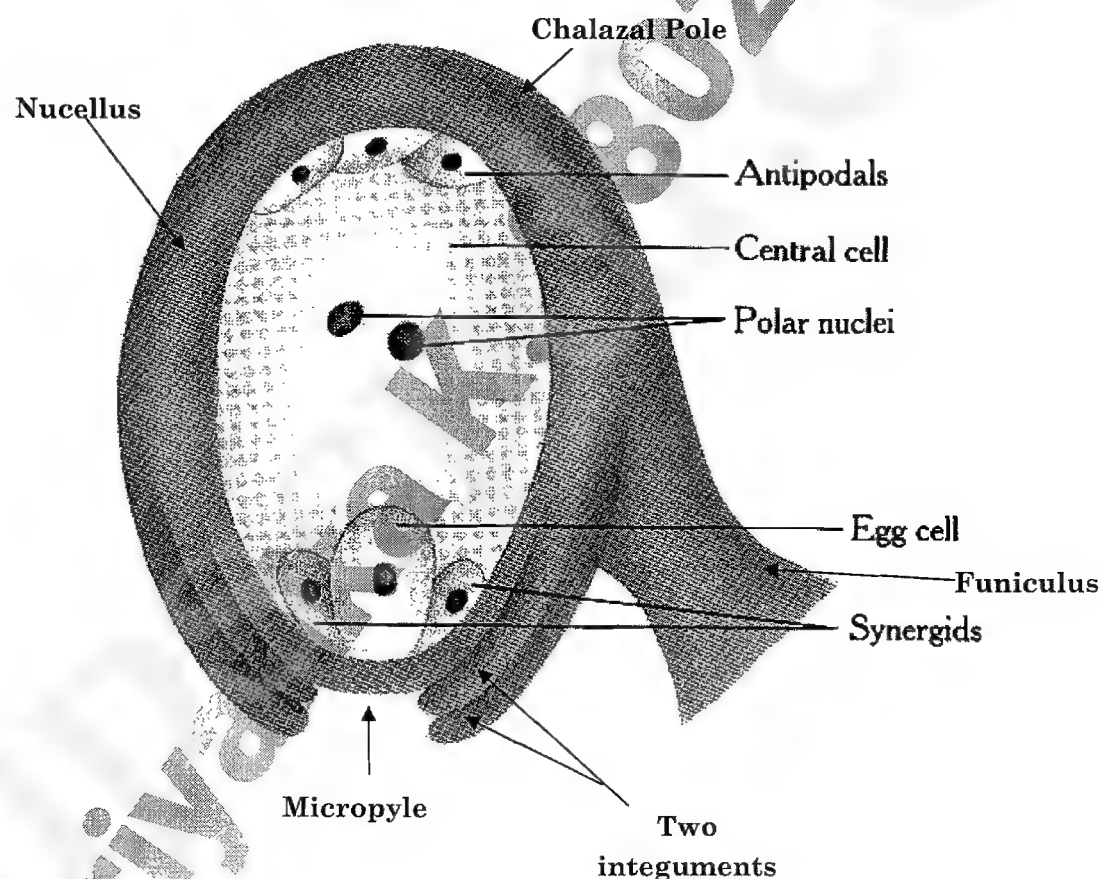


FIGURE 1: GENERAL ORGANIZATION OF A MATURE OVULE

Position of the ovule in female reproductive anatomy

A **carpel** is the female reproductive organ of a flower; the basic unit of the **gynoecium**. The carpel is differentiated into a basal fertile part (**ovary**) and an upper sterile part (**style & Stigma**). Thus, the parts of the carpel are:

- the **stigma** (*plural: stigmas*), usually the terminal (end) portion that has no epidermis and is fitted to receive pollen (male gametes); it is commonly somewhat glutinous or viscid;
- the **style**, a stalk connecting the *stigma* with the *ovary* below containing the transmitting tract, which facilitates the movement of the male gamete to the ovule; and
- the **ovary** (also called a **megasporophyll**) containing the female reproductive cell or

ovule. The ovule is attached to the placental wall of the ovary through the funiculus. In conifers and other gymnosperms, the ovules are borne on the surface of an ovuliferous (ovule-bearing) scale, usually within a cone, and are not enclosed by ovary

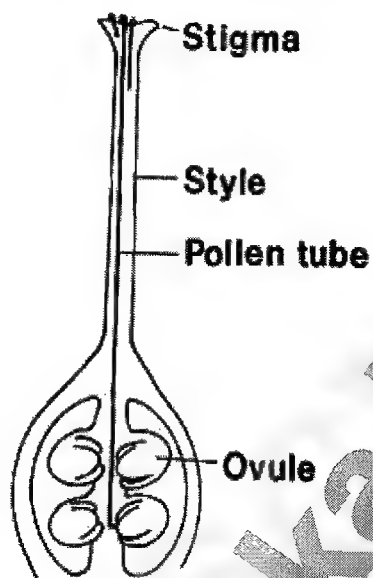


FIGURE 2: OVULES IN OVARY

Construction of the Ovule

The **ovule**, which represents the **megasporangium**, when mature, consists of one or two **integuments** surrounding the central **nucellus**, except at the apex where an opening, the **micropyle**, is left. In other words, the angiospermic ovule consists of a central body, the **nucellus**, and a basal stalk by which it is attached to the placenta, the **funicle**. The nucellus is protected and enclosed by one or two

sheaths; the **Integuments**. The opening in the integumentary sheath, where tip of the nucellus is exposed, is the **micropyle**.

The basal part of the ovule where nucellus, integuments and funicle merge is called **chalaza**.

The **embryo-sac**, formed in the nucellus, is the female gametophyte. In most angiosperms, the embryo-sac contains eight haploid nuclei. Of these eight nuclei, three at the chalazal end organize into **antipodal cells**, three at the micropylar end form **egg apparatus** and the two median nuclei are called **polar nuclei**. The egg apparatus has an **egg** and two **synergids**; the former represents the female gamete.

A brief description of the parts of the ovule

The various parts. of the ovule are as follows:

[I] Integuments

The angiospermic ovule has one (unitegmic) or two (bitegmic) integuments. Unitegmic ovules are characteristic of Gamopetalae whereas bitegmic ovules occur in Polypetalae, Archichlamydeae and Monocotyledons. Sometimes, as in *Liriosma*, *Olaix imbricate*, *Crinum*, etc., the ovule lacks integuments. Such ovules are called **ategmic**.

The two integuments arise independently; the inner integument usually precedes the outer integument. The two integuments may show various degrees of fusion between themselves and the nucellus. Usually the two integuments are fused at the chalazal end and are free towards the micropylar end, In some members of Cactaceae a prominent air space is present between the outer and inner integuments at the chalazal end.

It is generally believed that the unitegmic condition has arisen from the bitegmic condition either by fusion of the two integuments or due to abortion of one of the integuments. The unitegmic condition in Betulaceae, Ranunculaceae and some Papillionaceae is undoubtedly the result of fusion of two integuments, and in Salicaceae, it has been brought about by abortion of inner integument. Supernumerary integuments, i.e., more than two integuments have also been reported in some taxa. In *Asphodelus* and *Thantheina*, a third integument, called aril, arises from the base of the ovule and it covers completely the other two integuments. In many Euphorbiaceae, an outgrowth arises from the tip of the outer integument which turns backward and partially envelops the ovules. This outgrowth is called caruncle.

[II] Micropyle

It is a small pore present at the apex of the ovule, mostly formed by both the integuments. But in families like Podostemonaceae and Euphorbiaceae only the outer integument forms the micropyle. When both the integuments participate in the formation of micropyle, the part formed by the outer integument is known as **exostome** and by the inner integument as **endostome**.

Sometimes the exostome and endostome are not in a straight line and as such the micropyle appears zig-zag. In Leguminosae the exostome and endostome are at right angles to each other. Generally, the micropyle is filled with a mucilaginous substance secreted by nucellus or the integuments, which probably facilitates the entry of pollen tube in the ovule.

[III] Nucellus

Nucellus is a rounded or oval mass of thin walled parenchymatous cells, enclosed by integuments. In Polypetalae and Monocotyledons, the nucellus is massive and the sporogenous cell is deeply embedded in it. Such ovules are known as **crassinucellate**. This type of nucellus persists till the ripening of the seed. On the other hand, in

Gamopetalae the nucellus occurs only as a single layer around the sporogenous cell. Such ovules are called **tenuinucellate**. An extreme reduction of nucellar tissue is found in some members of Rubiaceae, where it is represented by a single celled cap over the sporogenous cell and in *Houstonia* it is altogether absent. Crassinucellate ovules are usually bitegmic and Tenuinucellate unitegmic.

However, Tenuinucellate and Crassinucellate conditions are not characteristic of any genus or species. Both tenui- and crassinucellate ovules occur commonly in *Butomus* and *Ophiopogon*.

The nucellus is usually confined within the limits of inner integument, but in Caryophyllaceae it extends into the micropyle. In *Codiaeum variegatum* (Euphorbiaceae) the nucellar tissue forms a **nucellar beak** which extends beyond the micropyle.

The nucellus is used as nutrition by the embryo sac or endosperm and is consumed almost completely by the time the endosperm matures. In Scitamineae and Piperaceae, however, the nucellus forms a special nutritive layer, called **perisperm**, around the embryo sac. This layer may persist even in the ripening seeds.

[IV] Hypostase and Epistase

These two structures within the ovule need special mention. In the basal part of the nucellus, in between the embryo sac and vascular bundles, there is a group of cells with lignified and suberized walls, which is called hypostase. These cells usually have poor cytoplasmic contents and deformed nuclei, but occasionally they may have dense cytoplasm and thin walls like glandular cells.

Hypostase is known to occur in many diverse families like Apiaceae, Loranaceae, Euphorbiaceae, Liliaceae and Elaeagnaceae, but its definite function is not yet known. Some consider that thick walled cells of hypostase limit the chalazal expansion of embryo sac, others regard it as a tissue responsible for maintaining water balance in dormant seeds. It is also considered as a glandular tissue which produces hormones required by the embryo sac.

In some tan such as *Costaria*, *Costus*, and *Agave* a few modified cells of the nucellus are present at the top of the embryo sac and these cells are referred to as epistase. The nature and function of **epistase** is not known.

[VI] Obturator

In families like Lamiaceae, Acanthaceae, Anacardiaceae, Magnoliaceae, Euphorbiaceae and Iridaceae, some uni- or multicellular hairs present in the basal part of the ovule collectively form the obturator. It may develop from the funicle or placenta. Obturator probably guides the pollen tube towards micropyle. It degenerates after fertilization.

Types of Ovule

Based upon the relative position of micropyle and chalaza at maturity, the following six types of ovules have been recognized.

[I] Atropous or Orthotropous ovules

The body of atropous ovule is upright with micropyle, chalaza and funicle falling in a straight line. Atrapous ovules are known to occur in some 20 families of angiosperms like Polygonaceae, Piperaceae, etc.

[II] Anatropous ovules

In this type, due to unilateral growth of funicle, the whole body of the ovule is inverted through 180°. As a result the micropyle comes close to the base of the funicle. The nucellus remains straight, i.e., micropyle and chalaza lie in one line and the funicle lies parallel to it. It is the most common type and occurs in several families of both dictyledons and monocotyledons.

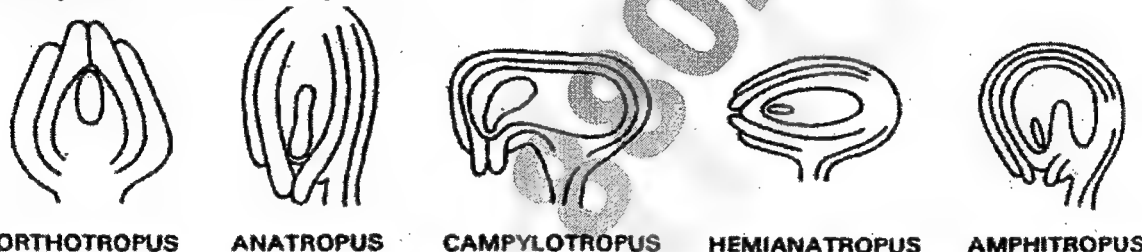


FIGURE 3: TYPES OF OVULES

[III] Campylotropous ovules

This type of ovule has a curved body but its curvature is less than that of the anatropous ovules. As such the micropyle and chalaza are not in a straight line and the funicle lies at right angles to the chalaza. Campylotropous ovules are known to occur in families like Capparidaceae, Chenopodiaceae, etc.

[IV] Hemi-anatropous or hemitropous ovules

In this type, the body of the ovule is turned through 90°, i.e., it is horizontally placed on the funicle. Hence the micropyle and chalaza are on a horizontal line and the funicle lies at right angles to this line. Hemi-anatropous ovules are found in the members of Ranunculaceae and Primulaceae.

[V] Amphitropous ovules

Such type of ovules have a pronounced curved body like that of anatropous ovule. But here the embryo-sac within the ovule also bends and becomes horse-shoe shaped. Amphitropous ovules are formed in the members of Loganiaceae, Alismaceae and Butomaceae.

[VI] Circinotropous ovules

(Not shown in the diagram).

In this type of ovules, the funicle is very long and forms as a complete circle around the body of the ovule. This type of ovules occurs in certain Plumbaginaceae (*Plumbago*) and Cactaceae (*Opuntia*, *Phyllocactus*).

4. Female gametophytes and their development

Megasporogenesis

At a very early stage of ovule development, a sub-epidermal cell at the apex of the nucellus differentiates as **archesporial initial**. Although all the hypodermal cells of the nucellus have the potential to develop into archesporial cells, usually a single cell differentiates as archesporial initial. It is distinguishable from the other cells by its conspicuous large size, dense cytoplasm and prominent nucleus.

Multicellular archesporium occurs in several families such as Fabaceae, Apiaceae, Salicaceae, Ranunculaceae, Poaceae and Liliaceae. A peculiar condition is found in *Casuarina*, which has a multicellular archesporium. The cells of the archesporium divide repeatedly to form longitudinal rows of cells. The average number of archesporial cells in *C. suberosa* is estimated to be about 300. A limited number of these cells form megaspore tetrads and the others remain sterile and eventually degenerate.

In tenuinucellate ovules the archesporial initial directly functions as megaspore mother cell. However, in crassinucellate ovules this cell divides by a periclinal wall forming an outer **primary parietal cell** and an inner **primary sporogenous cell**; the latter functions as **megaspore mother cell**.

The megaspore mother cell undergoes meiosis to form four haploid megaspores. As both the divisions of meiosis (meiosis I and meiosis II) are transverse to the long axis of the nucellus, a **linear tetrad** of megaspores is formed. A linear tetrad of megaspores is typical of most angiosperms, but occasionally the upper or lower cell of the dyad divides longitudinally instead of transversely, and this results in a T-shaped or inverted T-shaped tetrad.

Female Gametophyte (Embryo Sac)

The functional megaspore represents the first cell of the female gametophyte. It is mostly chalazal and grows mainly along the micropyle — chalazal axis.

In most of the angiosperms, as the functional megaspore grows, many small vacuoles appear in its cytoplasm which later join together to form a large vacuole. The nucleus undergoes three mitotic divisions and form eight nuclei.

The spindle of the first mitotic division is oriented along the vertical axis of the ovule. It is not followed by cytokinesis and the two daughter nuclei are separated by a large central vacuole. As the vacuole enlarges, one of the two daughter nuclei is pushed to the micropylar end and the other to the chalazal end. Both of these nuclei undergo two mitotic divisions, and as a result, four nuclei are formed at each pole. Subsequently these nuclei are reorganized; one nucleus from each group at a pole migrates to the centre of the cell. These two nuclei are called **polar nuclei**. The three nuclei left at the chalazal end are surrounded by walls and form **antipodals**. Of the three nuclei located at the micropylar end, one serves as **egg** or **female gamete** and the other two as **synergids**. These three together constitute the **egg apparatus**. The whole structure with two polar nuclei, three antipodals, one egg and two synergids is the mature **female gametophyte** or **embryo sac**.

Since this type of embryo sac develops from a single megaspore and has 8 nuclei, it is called **monosporic 8-nucleate embryo sac** or **Polygonum type** of embryo sac. It is the most common type of embryo sac and is found in about 85% of the flowering plants.

Types of Embryo Sacs

If we survey the angiosperm diversity, the following three types of embryo sacs are recognized based on:

- The number of megaspores taking part in the development of embryo sac
- The number of divisions occurring in the nucleus of the functional megaspore
- Organisation of nuclei in the mature embryo sac

The primary criterion used to classify embryo sacs is the number of megaspores contributing to the structure. Thus we have:

1. **Monosporic embryosacs**
2. **Bisporic embryosacs**
3. **Tetrasporic embryosacs**

(Please refer to the table below)

evolution

Angiosperm Embryo Sac Types



The most common type, about 80% angiosperms have this type of embryo sac. The Chalazal Megaspore forms the embryo sac. Composition: 3 celled egg apparatus, 3 antipodal cells and a Binucleate Central cell. The two nuclei of the central cell are called Polar Nuclei. [The mature structure is called 7 celled & 8 nucleated.] [As a matter of rule, all the monosporic embryo sacs have all the constituent cells genetically identical.]

The micropylar megaspore forms the embryo sac. After megaspore formation, 2 rounds of mitosis to arrive at a 4 celled configuration. Composition at maturity: 4 called, 4 nucleate. 3 called egg apparatus + 1 central cell with a single nucleus, NO ANTIPODALS. Characteristically seen in the family ONAGRACEAE and no where else. The only known exception is *Schisandra chinensis*. But there is one difference: in *Schisandra chinensis* the chalazal megaspore forms the embryo sac and not the micropylar megaspore as in true OENOTHERA TYPE.

Meiosis begins normally and after M-1, a dyad is formed. However, the micropylar cell of the dyad degenerates. The chalazal cell of the dyad survives and finishes M-2, forming only 2 megaspores. Both the megaspores undergo 2 rounds of mitosis each and form 8 nuclei. The final organization is just like POLYGONUM type. If the same process of development is shown by the micropylar cell of the dyad [that is formed after M-1], it will give rise to the ENDYMION TYPE embryo sac. Organization wise: Polygonum type, Allium type, Endymion type & Adoxa type are alike.

All the 4 megaspores contribute - hence genetically most diverse. [Monosporic embryo sac has one one genetic type of cells, Bisporic ones have 2 genetic types of cells and Tetrasporic embryo sacs have four genetic types of cells.] 4 megaspores undergo 2 rounds of mitosis each - generate 16 nuclei. Mature composition: 2 celled Egg Apparatus + 6 Antipodals [marginal in position] + 8 central cells.

All the 4 megaspores contribute - 4 megaspores undergo 2 rounds of mitosis each - generate 16 nuclei. Mature composition: 3 celled Egg Apparatus + 4 central cells + 3 groups of 3 cells each [one group is on chalazal end & two groups are on lateral margins].

All the 4 megaspores contribute - 4 megaspores undergo 2 rounds of mitosis each - generate 16 nuclei. Mature composition: 3 celled Egg Apparatus + 2 central cells + 11 cells on chalazal end behaving like antipodals.

After M-2, three megaspores fuse and form a triploid nucleus [first reported by Bambacini in 1928, hence also called BAMBACINI EFFECT]. The triploid nucleus undergoes 2 rounds of mitosis, generating 4 triploid cells. The haploid megaspore also undertakes two rounds of mitotic divisions to form 4 haploid nuclei. [So, post meiosis, only 2 rounds of mitotic divisions occur.] After cellular reorganization, the mature composition is: an egg apparatus of 3 haploid cells + 2 central cells [one haploid and one triploid] + 3 triploid antipodals.

After M-2, three megaspores fuse and form a triploid nucleus at the chalazal end. The triploid nucleus and the haploid megaspore undertake a single round of mitotic division each. After cellular reorganization, the mature composition is: an egg apparatus of 1 haploid cell + 2 central cells [1 haploid and 1 triploid] + 1 triploid antipodal.

All the 4 megaspores contribute - 4 megaspores undergo 1 round of mitosis each - generate 8 nuclei. Mature composition: 1 celled Egg Apparatus + 4 central cells + 3 groups of 1 cell each [one group is on chalazal end & two groups are on lateral margins].

All the 4 megaspores contribute - 4 megaspores undergo 1 round of mitosis each - generate 8 nuclei. Mature composition: 3 celled Egg Apparatus + 2 central cells + 3 antipodals: like the Polygonum type [for that matter like the Allium Type or Endymion Type]

THE EMBRYO SACS HAVE BEEN NAMED ACCORDING TO THE GENUS IN WHICH THEY WERE FIRST REPORTED & DESCRIBED.

Type	Megasporeogenesis				Megagametogenesis		
	Megaspore mother cell	Division I	Division II	Division III	Division IV	Division V	Mature embryo sac
Monosporic 8-nucleate Polygonum type							
Monosporic 4-nucleate Oenothera type						PLEASE NOTE: 3rd round of mitosis is needed only in Polygonum type	
Bisporic 8-nucleate Allium type							
Tetrasporic 16-nucleate Peperomia type							
Tetrasporic 16-nucleate Feneaea type							
Tetrasporic 16-nucleate Drusa type							
Tetrasporic 8-nucleate Fritillaria type							
Tetrasporic 4-nucleate Plumbagella type							
Tetrasporic 8-nucleate Plumbago type							
Tetrasporic 8-nucleate Adoxa type							

Monosporic embryo sacs

A monosporic embryo sac develops from a single megaspore and as such, all the nuclei present in this type of embryo sac are genetically alike. Monosporic embryo sacs are of the following two types:

1. **Monosporic 8-nucleate or Polygonum type:** This type of embryo sac develops from the chalazal megaspore. Its nucleus divides thrice to form eight nuclei. This type is generally referred to as **normal type of embryo sac**. It is also called **Polygonum type** as it was first time described in *Polygonum divaricatum* by Strassburger (1879).
2. **Monosporic 4-nucleate or Oenothera type.** This type of embryo sac usually develops from the micropylar megaspore. The megaspore nucleus divides twice and forms only four nuclei; of these three organise into egg apparatus and the fourth functions as polar nucleus. Thus, Oenothera type of embryo sac does not have any antipodals. This type is characteristic of the family Onagraceae.

Bisporic embryo sacs

The bisporic embryo sac develops from one of the two dyads formed because of the first meiotic division (meiosis I) of megaspore mother cell. One of the dyads degenerates. Both the nuclei arising from the functional dyad take part in the formation of embryo sac. Each nucleus undergoes two mitotic divisions and as such, the mature embryo sac is 8-nucleate. The eight nuclei are organized into antipodals, egg apparatus and polar nuclei as in Polygonum type of embryo sac. In this type of embryo sac the 4 nuclei derived from one megaspore nucleus are genetically different from the other four derived from the second megaspore nucleus.

Based on the position of functional dyad the following two types have been recognized in bisporic embryo sacs.

1. **Allium type:** This type develops from the chalazal dyad.
2. **Endymion type:** This type develops from the micropylar dyad.

Tetrasporic embryo sacs

Sometimes meiotic division of the megaspore mother cell is not accompanied by cytokinesis and hence all the four haploid nuclei lie in a single cell called **coeno-megaspore**. All the four nuclei of coeno-megaspore participate in the formation of embryo sac. This type of embryo sac is called **tetrasporic** and it is genetically more heterogeneous than the bisporic type of embryo sac.

On the basis of :

- the position of haploid nuclei in the coeno-megaspore

- the number of times these nuclei divide
- organisation of nuclei in the mature embryo sac

the following types of tetrasparic embryo sacs have been recognised.

1. **Adoxa type.** It has 8 nuclei which are formed by the mitotic division of the four haploid nuclei of the coeno-megaspore. **The arrangement of the 8 nuclei in the embryo sac is the same as in Polygonum type.** This type of embryo sac occurs in *Adoxa*, *Sambucus*, *Ulmus*, *Tulipa*, *Erythronium*, etc.

2. **Plumbago type.** In this type, of the four haploid nuclei of the coeno-megaspore, one migrates to the micropylar end, one to the chalazal end and two on two lateral sides. Each of these four nuclei divides again, with the result four groups of two nuclei each are formed. One of the nucleus from each group moves to the centre of the cell and these form four polar nuclei. The remaining nucleus at the micropylar end is cut off by a membrane and forms the egg. **There are no synergids.** The other three nuclei (i.e., one chalazal and two lateral) usually disappear but occasionally they too may be cut off by membranes and appear as accessory egg cells.

The Plumbago type of embryo sac is thus characterised by the absence it synergids and antipodals. This type of embryo sac is known to occur in the Plumbaginaceae.

3. **Penaea type.** In this type, the four haploid nuclei of the coeno-megaspore undergo two successive mitotic divisions forming 16 nuclei. These nuclei arrange themselves in four groups of four each, one at the micropylar end, one at the chalazal end and one each an the two lateral sides. Now one nucleus from each group migrates to the centre, and these four nuclei in the centre form polar nuclei. The three nuclei at the micropylar end are cut off by membranes and form the egg apparatus. The remaining three groups of nuclei (one chalazal and two lateral) degeherate. **Thus at maturity the Penaca type of embryo sac has an egg apparatus and four polar nuclei. There are no antipodals.**

In Penaea type of embryo sac a highly polyploid (5x) primary endosperm. nucleus is formed after double fertilization. In addition to the family Penaeaceae, this type of embryo sac is also found in the Malpighiaceae and Euphorbiaceae.

4. **Peperomia type.** The four haploid nuclei of coeno-megaspore undergo two successive mitotic divisions forming 16 nuclei. Of these, two nuclei at the micropylar end form egg and a synergid; eight fuse in the centre of the cell to form a polar nucleus and the remaining six which are cut off by membranes at the chalazal end may be compared with antipodals. **Thus the egg apparatus of Peperomla type is characterised by a single synergid.** This type of embryo-sac occurs in members like *Peperomia* and *Gunnera*.

5. Drusa type. Like Penaea and Peperomia type this is also a 16- nucleate embryo sac. In the mature embryo sac three nuclei form egg apparatus, two act as polar nuclei and the remaining 11 nuclei are cut off by membrane and form antipodal cells. **Thus Drusa type is characterised by large number of antipodals.**

In Drusa type of embryo sac the number and organisation of nuclei may vary due to irregularity in the divisions. This type of embryo sac is found in *Drusa*, *Rubia*, *Chrysanthemum*, *Ulmus*, etc.

6. Fritillaria type. The four haploid nuclei of the coenomegaspore arrange themselves in two groups — three at the chalazal end in the form of a triploid nucleus and one at the micropylar end of the cell. The triploid chalazal as well as the haploid micropylar nucleus undergo two mitotic divisions and as a result four triploid nuclei are formed at the chalazal end and four haploid at the micropylar end. In the mature embryo sac three haploid nuclei organize into egg apparatus, three triploid into antipodals and remaining one haploid and one triploid nuclei move to the centre where they fuse to form a tetraploid polar nucleus. **Thus, in this type, the antipodal nuclei are triploid and the polar nucleus is tetraploid.** This type of embryo sac is found in members like *Fritillaria*, *Lilium*, *Piper* and *Gaillardia*.

7. Plumbagella type. The initial development of this type of embryo sac is similar to Fritillaria type and a triploid nucleus is formed at the chalazal end and a haploid at the micropylar end. Each of these nuclei undergoes a single mitotic division and form two groups of two nuclei each. One triploid nucleus from the chalazal end fuses with a haploid nucleus at the centre of the sac to form a tetraploid polar nucleus. One haploid nucleus at the micropylar end forms the egg and one triploid nucleus at the chalazal end the single antipodal. There are no synergids. **Thus in the mature embryo sac of Plumbagella type the egg apparatus consists of a single cell which represents the egg.**

All individuals of a species may not necessarily show only one type of embryo sac. One may come across more than one type of embryo sac within the same species. The number of nuclei in the embryo sac have been found to be affected by change in temperature; a high temperature (26—27°C) accelerates nuclear divisions whereas a low temperature (15— 19°C) inhibits this process.

5. Pollination

Introduction to pollination

Pollination is the process by which pollens are transferred from a dehiscent anther to a receptive female structure of seed plants. In gymnosperms, the female receptive structure is the micropyle of the ovule. In angiosperms, this structure is the stigma of the carpel. Pollination enables fertilization and sexual reproduction in seed plants.

Types of pollination

In angiosperms, pollination is of two types.

1. Cross-pollination
2. Self-pollination

Cross-pollination

It is also called *alogamy*. It occurs when pollen is delivered to a flower from a different plant. Plants have different morphological and physiological mechanisms to ensure cross-pollination. Some of these mechanisms include:

1. Dicliny or unisexual plants. Flowers may be only male or female and are borne on different plants, e.g. palms, papaya.
2. Heterostyly in *Primula vulgaris*, *Lythrum* and *Oxalis*. There are flowers in which the length of style and the position of anthers are different. One type bears stamens at a higher level and style is short. The other form bears long style and the stamens are at a lower level. In both these cases cross pollination by insects is the only method.
3. Herkogamy in *Gloriosa* and Caryophyllaceae. In this condition male and female sex organs are so placed in a flower that the pollen grains from the anther are unable to reach the stigma in the same flower.
4. Dichogamy in *Saxifraga*, *Impatiens* and *Aristolochia*. In this case, the male and female parts of the flower mature at different times.
5. Self sterility in *Petunia*, many Solanaceae, Brassicaceae and Poaceae members. Here, pollen grains of a flower fail to fertilize the flower of the same plant.

Cross pollination is an advanced behaviour as it increases genetic variability.

Self-pollination

It occurs when pollen from one flower pollinates the same flower or other flowers of the same plant. It is a primitive type of pollination. It possibly evolved when pollinators were not reliable vectors for pollen transport. It is mostly seen in short-lived annual species and plants that colonize new locations.

Self pollination is of three types.

1. Autogamy, where pollen moves to the female part of the same flower
2. Geitonogamy, when pollen is transferred to another flower on the same plant.
3. Cleistogamy is self-pollination that occurs before the flower opens.

Agents of pollination

There are two fundamental types of pollination agents.

1. Abiotic agents
2. Biotic agents

Abiotic agents of pollination

There are two abiotic agents of pollination.

1. Wind
2. Water

Wind mediated pollination

Wind mediated pollination is also known as *anemophily*. It is considered a primitive mode of pollination because it is a non-directional and wasteful process. Nearly all gymnosperms show anemophily but very few angiosperms have this type of pollination. Some examples include *Mercurialis annua*, *Zea mays* etc. Anemophilous angiosperms have small flowers, produce small and light pollen grains but in great numbers and have the pollen walls dry and smooth to facilitate wind mediated pollination.

Water mediated pollination

This type of pollination is also called *hydrophily*. The angiosperms adapted to hydrophily have small flowers with reduced floral envelopes. There are two types of hydrophily.

1. Hyphydrophily: In this, the pollen grains are dispersed below the water surface. Examples include *Ceratophyllum*, *Najas*, *Zostera* etc.
2. Ephydrophily: In this, the pollen grains are dispersed above the water surface, though the plant may be mostly submerged (but, the flower develops on the water surface). Example includes *Vallisneria spiralis* etc.

Biotic agents of pollination

There are three main abiotic agents of pollination.

1. Insects
2. Birds
3. Bats

Insect mediated pollination

Insect mediated pollination is also called Entomophily. This is the most common type of pollination for angiosperms in the tropics. The insects involved in this process mainly include the bees, Lepidoptera (like butterflies and moths), flies and beetles. Among these, the bees are most important (*mellitophily*). It is estimated that about 80% of entomophily is actually carried out by bees.

Entomophily is also the most advanced mode of pollination, because it is highly specific. Specificity of the process arises from the fact that a certain insect species visits only one or a limited number of plant species. This specificity minimizes the loss of pollen grains.

Entomophilous plant species have evolved mechanisms to attract the insects, e.g., brightly-colored or scented flowers, nectar, or appealing shapes and patterns. Some examples include the following.

1. Plants develop nectar to attract bees. The bees also collect pollen grains for feeding their larvae.
2. Flowers become brightly coloured to attract bees. The bees do not recognize red colour. Bee pollinated flowers are mostly yellow.
3. Some flowers have distinct markings on the petals. These



FIGURE 4: TURN-PIPE FLORAL MECHANISM IN SALVIA

markings are called *honey guides*. They serve to attract the bees by guiding them towards the source of nectar.

4. Moths visit the flowers during night. Hence moth pollinated flowers are generally white or pale but they have strong smells, especially being exuded in night.
5. Beetle and fly pollinated species often release unpleasant odour due to some amine containing compounds in order to attract the pollinators. Examples include *Rafflesia*, *Arum*, *Aristolochia*, *Magnolia*, lily and wild rose.
6. *Salvia* has developed the *turn-pipe floral mechanism* to deposit pollen grains on the insect body (Fig. 1)
7. *Aristolochia* and *Ceropegia* have developed the *Fly-trap mechanism* of pollination.
8. *Ophrys speculum*, an orchid, has developed its flowers in a way that it resembles the female insects of the species *Colpa aurea* in both shape and smell. This attracts the male insects of the species to these flowers and uses them as pollinators.
9. *Calotropis* has developed a structure called gynostegium to disperse its pollinium.

Bird mediated pollination

This type of pollination is also called *Ornithophily*. It has evolved from insect pollination. It is well distributed in the tropics and on some island chains.

The ornithophilous plants have colourful (often red) flowers with long tubular structures holding ample nectar and such arrangements of the stamen and stigma which ensure contact with the pollinator. Birds involved in ornithophily are nectarivores with brushy tongues, long beaks, and light enough to perch on the flower structures.

Examples of bird pollinated flowers include *Banksia*, *Nicotiana glauca*, *Callistemon*, *Butea*, *Bombax*, *Spathodea*, *Fuchsia* etc. The birds involved in ornithophily include sun birds, hummingbirds etc.

Bat pollinated flowers

It is also called *Cheiropterophily*. It is common in the tropical parts of the world. About 500 tropical plant species are completely, or partially, dependent on bats for pollination (Heithaus 1974).

Plants pollinated by bats often have white or pale nocturnal flowers that are large and bell shaped. Many of these flowers have large amounts of nectar, and emit a smell that attracts bats, such as a strong fruity or musky odour. Bats use certain chemical signals

to locate food sources. They are attracted to odours that contain esters, alcohols, aldehydes, and aliphatic acids. Bat pollinated plant species include *Mucuna gigantea*, *Adansonia digitata*, *Kigelia africana*, some varieties of mango in the Caribbean islands etc.

Pollination by other animals

Apart from the principal methods outlined above, pollination is also carried out in specific angiosperms by monkeys, marsupials, lemurs, bears, rabbits, deer, rodents, lizards and other animals. Some plant species like *Arctium*, *Acaena*, *Quararibea*, *Combretum* and *Galium aparine* use alternative modes of zoophily. A symbiotic relationship exists between the African Lily, *Massonia depressa* and its pollinator species rodents. The lizard *Hoplodactylus* is attracted by nectar on flowers and helps in the pollination of flowers of *Metrosideros excels*.

Importance of pollination in agriculture

Pollination management is one of the most fundamental steps in hybridization experiments. Hybridization is effective pollination between flowers of different species, or between different breeding lines or populations in order to obtain a set of desirable traits in a single plant line. In agriculture and horticulture pollination management, a good pollen source is a plant that provides compatible, viable and plentiful pollen and blooms at the same time as the plant that is to be pollinated or has pollen that can be stored and used when needed to pollinate the desired flowers.

6. Fertilization

When the pollen grain is shed from the anther it has two cells— a generative cell and a tube cell. The generative cell eventually forms two male gametes. In angiosperms the female gametophyte (embryo sac) is situated in the ovule, at a distance from the stigma. Therefore, in order to transport male gametes into the female gametophyte, the pollen produces a pollen tube on the stigma. The pollen tube with two male gametes grows down through the style and enters the ovule usually through the micropyle. It releases two male gametes into the embryo sac. One of the male gametes fuses with the egg and forms the zygote. **This fusion of male and female gametes is known as fertilization.** The second male gamete fuses with the two polar nuclei (or secondary nucleus if the two have already fused) and forms a **triple-fusion nucleus**, called **primary endosperm nucleus**.

The sequence of events leading to fertilization in angiosperms is described below.

Germination of Pollen Grains

Once the pollen grain has landed on the receptive stigma, its germination starts. One of the requirements for germination of the pollen grain is that it should adhere to the stigmatic surface. The stigma shows a number of adaptations to achieve this. In plants like *Petunia hybrida*, *Strelitzia reginae* and *Zea mays* the stigma secretes a sticky and oily exudate and pollen grains adhere to this stigmatic fluid. In *Brassica* pollen grains stick to the stigmatic papillae because of a 'melting together reaction' with the waxy coating of the cuticle brought about by cutinase enzyme. In *Cosmos* pollen grains are attached to the stigmatic surface by mucilaginous strands.

In some 80 families of angiosperms the stigma has short papillose outgrowths. The surface of these papillose outgrowths is made of hydrophilic proteins, which keep it moist. This helps in the hydration of pollen grains. The stigmatic papillae collapse after pollination and a watery substance, formed by degeneration of their cytoplasm, helps in pollen germination.

Besides stigmatic surface, the pollen can also germinate in the anther sac as in cleistogamous flowers, on the style, if the stigma has been chopped off, and on the surface of petals.

The stigmatic fluid, secreted on the stigma, contains lipids, resins, sugar, etc., and thus provides a suitable medium for the germination of pollen grains. Cytochemical studies have shown the presence of many hydrolytic enzymes such as acid and alkaline phosphatase, ribonuclease, esterase and amylase in the intine, principally below the

aperture region of the pollen grain. These enzymes play a significant role in the process of pollen germination.

Usually pollen grains are monosiphonous, i.e., each pollen grain produces only a single pollen tube, but in Campanulaceae, Cucurbitaceae and Malvaceae they are polysiphonous. In *Althea rosea* as many as 10 pollen tubes from a single pollen grain have been observed, and in *Malva neglecta* the number goes up to 14. Where more than one pollen tubes are formed, only one which carries pollen nuclei grows up to the ovule and the others degenerate at different stages of development. In the Orchidaceae and Asclepiadaceae, where pollen grains are grouped in pollinia, a large number of pollen grains may germinate simultaneously to form several pollen tubes. The pollen tubes are branched in Amentiferae.

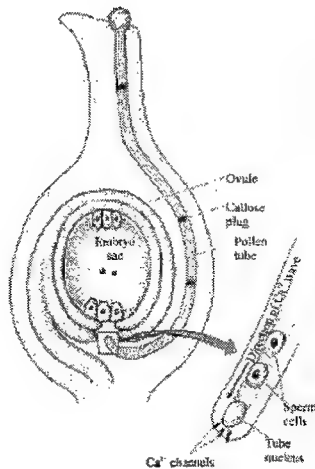
An important factor which determines germination of pollen grains on the stigma is pollen stigma interaction, in fact, at the stigmatic surface pollen grains are first recognized and then allowed to hydrate and germinate. In self-incompatible mating pollen grains carry certain 'factors' in their exine which produce rejection-response in stigmatic surface.

Growth of Pollen Tube

The growth of the pollen tube down through the style depends upon internal structure of the latter.

- **Hollow style:** The styles of some flowers like *Lilium* and *Ribes* are hollow and the mucilagenous secretions of the cell lining of the stylar canal help in the passage of pollen tube through the style.
- **Solid style:** Mostly the style is solid and hence passage of tube involves the secretion of tissue dissolving enzymes (pectinase) by the growing tip of the tube. Contrary to this, in *Oenothera*, *Petunia* and *Datura* the pollen tube grows through the intercellular spaces in the style.

As the pollen grain germinates, its entire contents move into the pollen tube. The growth of the pollen tube is primarily restricted to its tip where most of the cytoplasm is concentrated. The remaining part of the pollen tube and the pollen grain are occupied by vacuoles. The cytoplasm is restricted to the tip of the pollen tube by callose plug. As the pollen tube grows, new plugs are formed continuously and as such several callose plugs can be seen at short interval in a long pollen tube. These plugs divide the pollen tube into small segments. The plugs originate as small rings which grow centripetally and finally seal the tube. The extreme tip of the pollen tube, which appears as a hemispherical transparent area under light microscope, is known as cap block. There is some correlation between the appearance of cap block and the growth of the pollen tube. The tube grows so long as the cap block exists and its growth ceases as soon as the cap block disappears.



The wall of the pollen tube is made of cellulose and pectin. The amount of pectin is maximum in the cap block area and gradually decreases towards the proximal end of the tube. The cytoplasm of the pollen tube, just behind the cap block has numerous mitochondria, Golgi-bodies, endoplasmic reticulum and lipids. In the cap block region the cytoplasm shows abundant Golgi vesicles which are rich in polysaccharides and RNA.

The growth of the pollen tube in the style is always directed towards the ovary. Such unidirectional growth is perhaps due to hydrotropic, chemotropic and mechanical factors. There are enough evidences to show that chemotropic substances leach out of the filiform

apparatus of synergids and attract the pollen tube towards embryo sac with waves of Calcium ions. Accumulation of metabolically active substances at the tip of the pollen tube establishes a physiological polarity, and this also contributes to unidirectional growth of the pollen tube.

Entry of Pollen Thin into Ovule

On reaching the ovary, the pollen tube grows towards one of the ovules. It may enter the ovule through one of the following three routes:

[I] Porogamy

When the pollen tube enters into the ovule through micropyle, it is known as **porogamy**. This is the most common mode of pollen tube entry into the ovule. It is believed by some that substances secreted by filiform projections of synergids are responsible for porogamy. But this view does not hold good as porogamy is found even in those cases where embryo sacs are without synergids.

[II] Chalazogamy

When the pollen tube enters the ovule through its chalazal end, it is called chalazogamy. It is common in many Amentiferous taxa such as *Betula*, *Juglans regia*, *Casuarina*, *Ostrya* and *Fterocatya*.

[III] Mesogamy

When the pollen tube enters the ovule through integuments, the condition is described as **mesogamy**. The pollen tube generally enters the ovule through the side of the integument nearest to the placenta. *Alchimella*, *Cucurbita* and *Populus* are some examples of mesogamy.

Entry of Pollen Tube in the Embryo-sac

Irrespective of the place of entry into the ovule, the pollen tube always enters the embryo-sac through the micropylar region. It may enter the embryo sac via one of the following routes:

- (i) between the egg cell and one of the synergids without destroying the latter
- (ii) between the wall of the embryo sac and one or both the synergids
- (iii) between the two synergids without destroying either of them
- (iv) directly penetrates one of the synergids

Recent studies have confirmed that synergids not only have an important role in determining the entry of pollen tube in the embryo sac but they also affect dissemination of male gametes in the embryo sac.

Movement of sperms toward egg and polar nuclei

The contents of pollen tube are released in one of the synergids, and as the egg cell and synergids are in close contact, sperms do not have to travel long. Although fertilization is basically fusion of male and female gametes, the pollen tube never directly enters the egg cell. In some embryo-sacs, where synergids are absent (e.g., *Plumbago* and *Plumbagella* types), the pollen tube penetrates the embryo sac in between the wall of the embryo sac and egg cell. The sperms show amoeboid movements, one of the male gametes moves towards the egg and the other to the polar nuclei. The factors or forces, which propel one of the sperms into the egg and the other into the central cell with remarkable regularity and precision, are not properly known. Only one of the two sperms fuses with the egg. The receptiveness of the egg is lost once a sperm has fused with it.

Fusion of Gametes

Fusion of one of the male gametes with the egg is known as **syngamy** or **true fertilization**. It results in the formation of a **diploid zygote**. The union of second male gamete with the polar nuclei results in the formation of primary endosperm cell and constitutes **double fertilization**. As most of the embryo sacs have two polar nuclei, double fertilization involves fusion of three nuclei, i.e., **triple fusion**. The contact between one of the male gametes and the egg is established earlier than between the second male gamete and polar nuclei. But the primary endosperm is formed earlier than the zygote. It is perhaps because the cytoplasm of the central cell is more active than the egg.

Related topics

Interval between pollination and fertilization

The time gap between pollination and fertilization is from 2 hr to 12 days or even more. Syngamy and triple fusion usually begin simultaneously; wherever these processes are disjunctive, it is mostly the triple fusion which comes off earlier. The actual process of nuclear fusion is also more brisk in the central cell than in the egg.

X-bodies

After the discharge of pollen tube contents into the embryo sac, two darkly stained bodies have been observed in the synergids penetrated by the pollen tube, or in the vicinity of the egg apparatus. These bodies, called **X-bodies** by S.G. Nawaschin, have been variously interpreted as decomposition products of vegetative nucleus, supernumerary male gametes, or discarded cytoplasmic sheaths of the male gametes. Ultrastructural studies and refined staining techniques have, however, shown that x-bodies contain DNA, and are, therefore, nuclei. One of the x - bodies represents a degenerated vegetative nucleus and the other, the remains of synergid nucleus.

Polyspermy

In many taxa more than two sperms (male gametes) are released in an embryo sac. This condition is referred to as polyspermy. It may arise either due to the presence of more than two sperms in a pollen tube or due to the entry of more than one pollen tube in an embryo sac. Normally an embryo sac receives only a single pollen tube and only one sperm fuses with the egg, but polyspermy may bring about fertilization of egg by more than one sperm. Occasionally supernumerary sperms may fertilize synergids or antipodal cells. This may result in the formation of more than one embryos in an embryo sac. If the embryo sac receives two or more pollen tubes and the sperms fusing with the egg and polar nuclei belong to different pollen tubes, the condition is described as **heterofertilization**. Processes like polyspermy and heterofertilization are not very frequent in nature and may be considered aberrations only.

7. Plant in-vitro (test-tube) fertilisation

Introduction

In vitro fertilization (IVF) is an experimental process by which egg cells are fertilized by sperm outside the mother's body that is *in vitro*.

With respect to plants, the process involves:

- Isolation of eggs (either extracted or within the ovule) from the female flower
- Letting sperm (arising from cultured pollen) fertilize the eggs in a fluid medium.

The fertilized egg (zygote) is then cultured to regenerate the whole plant.

Need for plant in-vitro fertilization

The techniques of in-vitro pollination and fertilization provide powerful tools for studying fundamental aspects of pollen-pistil interaction and fertilization, and effective manipulation of these processes. The need for plant IVF arises due to the following reasons.

- (1) Plant IVF is a great tool for understanding the cellular-physiological basis of plant fertilization. This is a poorly understood aspect of reproduction in higher plants. The reason for this is that fertilization in flowering plants takes place deep inside the ovule, which imposes technical problems for both descriptive and experimental studies.
- (2) Plant IVF is an efficient method to overcome many types of compatibility barriers including the major mechanisms of both SSI and GSI.
- (3) Plant IVF enables effective experimentation on fertilization which can lead to rapid progress in applied aspects of fertilization – such as obtaining viable crosses which are otherwise not possible by any sexual method.

History of plant in-vitro fertilization

Although a beginning was made during the 1960s, it is only in 1990s that true in-vitro fertilization using isolated sperms and eggs has been achieved for plant species.

The earliest step in this direction was taken during the 1960s, when the technique of pollination of cultured ovules was standardized in the laboratory of Dr. P. Maheshwari, Department of Botany, University of Delhi. The method was standardised in the members of the family Papaveraceae.

The process of plant in-vitro fertilization

Mainly there are two principal approaches to plant in vitro fertilization.

1. In-vitro pollination (the older method)
2. In-vitro fertilization of extracted gametes (modern method)

1. In-vitro Pollination

The technique of in-vitro pollination involves aseptic pollination of cultured pistils or ovules to achieve pollen germination, pollen tube growth and its entry into the embryo sac, double fertilization and subsequent development of embryo, endosperm and seed. In this, there are two methods.

Pollination of cultured pistils

Pollination of cultured pistils and their growth in-vitro into mature fruits provide a very convenient technique of IVF.

It has been successfully achieved in species of *Nicotiana*, *Petunia* and *Antirrhinum*.

In this technique:

1. The pistils were surface sterilized
2. Cultured on a simple nutrient medium
3. Pollinated with fresh pollen collected aseptically

Pollen grains in compatibly pollinated pistils germinated, pollen tubes grew through the style, entered the ovary and effected fertilization.

Such cultures developed into normal mature fruits in about 3 weeks.

Limitations:

Pollination of cultured pistils was not effective in overcoming sexual incompatibility because the zone of inhibition, namely the stigma and style, was intact.

Pollination of cultured ovules

This was standardized during the 1960s on the members of Papavaraceae by Dr. P. Maheshwari.

It involves:

1. Injecting the pollen grains (suspended in a suitable medium) directly into the ovary
2. Achieving pollen germination, entry of pollen tubes into ovules and fertilization.

Viable seeds consequent to intra-ovarian pollination have been obtained in *Papaver somniferum*, *P. rhoeas*, *Argemone mexicana* and *A. ochroleuca*.

The intraovarian pollination technique has also been applied to achieve interspecific hybridization between *A. mexicana* and *A. ochroleuca*.

Limitations:

1. It is not suitable for species in which there is not enough space in the ovary to inject pollen suspension.
2. Also, in species in which a sugar (usually sucrose) is required for pollen germination, injection of pollen suspension in sugar solution may render the ovary prone to bacterial and fungal infections.

In spite of these limitations, pollination of cultured ovules is an important method for overcoming sexual self-incompatibility in many cases.

2. In-vitro Fertilization of extracted gametes

This method relies upon protoplast technology and somatic hybridization.

The pioneering success of fusing isolated egg and sperm cells in-vitro was accomplished in *Zea mays* by Kranz and Dresselhaus in 1996 at the University of Hamburg.

This method involves:

1. *Sperm isolation*: Basically two procedures were used to isolate the sperms from pollen grains / tubes:
 - a. Mechanical method (grains / tubes are ruptured mechanically); and

- b. Osmotic shock method (pollen grains / tubes are allowed to rupture in a hypotonic solution)
2. *Embryo sac isolation*: Embryo sacs are generally isolated by combining enzymic maceration of ovules with microdissection. Subsequently the experimenter isolates protoplasts from constituent cells of the embryo sac (egg, synergids and central cell).
3. *Fusion of gametes*: Fusion of gametes is performed under the microscope in microdroplets of fusion medium. Isolated egg and sperm cells held in microcapillaries are transferred to the fusion droplets by using a computer-controlled dispenser. The egg and sperm cells are aligned electrophoretically or mechanically with microneedles. Fusion is induced by giving one or a few short pulses of direct current. Fusion is completed in less than 1 second and karyogamy occurs in less than 1 hour from fusion.

The in-vitro-formed zygotes are then cultured, giving rise to globular structures, proembryos and transition phase embryos comparable to seed embryos in vivo. The eventually form fertile plants.

Applications of in-vitro Pollination and Fertilization

1. In-vitro pollination and fertilization provide very effective techniques in fundamental and applied areas of fertilization and seed development.
2. Pollination of cultured pistils is simple and can be used for a number of experiments on seed and fruit development.
3. The method allows overcoming several types of compatibility barriers.
4. The intra-ovarian pollination method helps in studies of induced parthenogenesis and in mutation research because in this method only the female partner (ovules) can be treated with any chemical or physical factor.
5. Use of pollen tube cultures for in-vitro pollination offers a convenient method of treating only the male partner (gametes in pollen tubes) with physical and chemical mutagens.

8. Endosperms

An introduction to endosperm

In seed plants, tissue that surrounds and nourishes the embryo in the seed is known as **endosperm**.

In gymnosperms, the endosperm is an unmodified female gametophyte. Therefore, it is a haploid tissue.

In angiosperms, however, the initiation of endosperm is the result of *triple fusion*. This is a definitive characteristic of angiosperms. It requires the fusion of at least one (but mostly two) nucleus in the embryo sac central cell with a sperm nucleus.

This is a critical structure for normal embryo development. Therefore, all angiosperm families *except three* (Orchidaceae, Trapaceae, and Podostemaceae) form endosperm tissue in the seed.

The seeds where endosperm develops, there is a variation in its persistence. There are two patterns observed:

1. In some seeds the endosperm gets completely absorbed at maturity (*e.g.*, pea and bean). Such seeds are called **exalbuminous**.
2. In other seeds, endosperm is present until germination (*e.g.*, wheat, castor bean). These seeds are called **albuminous**.

Features of endosperm

1. Endosperm is a unique tissue in angiosperms, arising out of Triple Fusion. In most of the angiosperm, the endosperm cells are triploid. Endosperm ploidy may however vary according to the number of polar nuclei in the central cell of embryo sac. For example, in the *Oenothera* type of embryo sac, where there is only one polar nucleus, the endosperm is diploid (2n) and the *Peperomia* type of embryo sac has eight polar nuclei, hence the endosperm is 9n. In general, the endosperm ploidy can vary widely from diploid (2n) to 15n.
2. The cells of the endosperm usually do not contain chlorophyll, but in some species of *Crinum*, *Raphanus* and *Viscum* chlorophyll is present in the cells of the endosperm.

3. Endosperm normally develops faster than the embryo. It is characterized by high levels of auxin and cytokinin action.
4. The endosperm cell walls are thin, mainly made of a single pectocellulosic layer.
5. In some species, endosperm develops polytene chromosomes to increase the levels of protein synthesis.
6. In most cases, the endosperm develops haustoria to absorb nutrition from neighbouring tissues.

Development of endosperm

Endosperm is formed when the two sperm nuclei inside a pollen grain reach the interior of an embryo sac or female gametophyte. One sperm nucleus fertilizes the egg, forming a zygote, while the other sperm nucleus fuses with the two polar nuclei at the center of the embryo sac, forming a primary endosperm cell. Its nucleus is often called the *triple fusion nucleus*.

This cell created in the process of double fertilization develops into the endosperm. Because it is formed by a separate fertilization, the endosperm constitutes an organism separate from the embryo.

Types of endosperms

On the basis of their development, the following three types of endosperm have been recognized:

1. Nuclear endosperm
2. Cellular endosperm
3. Helobial endosperm

Nuclear Endosperm

In the development of nuclear endosperm the first few divisions of the primary endosperm nucleus are not accompanied by cell wall formation and nuclei thus produced remain free in the cytoplasm of the embryo sac. The wall formation occurs subsequently as in *Glycine max* and *Arachis hypogaea* or the nuclei remain free indefinitely as in *Limnathes douglasii*, *Acer pseudoplatanus* and *Myricaria germanica* etc.

It has been suggested that in those plants where embryo grows rapidly since the outset, the endosperm tends to be non-cellular.

The first few divisions of the primary endosperm nucleus are synchronous but the later divisions are irregular. Generally, hundreds of free nuclei are found along the periphery in the embryo sac, as seen in *Primula*, *Malva*, *Mangifera*, *Citrus*, *Arachis*, etc.

All endosperm nuclei are not essentially of the same size. Usually the nuclei at the chalazal end are larger and those at the micropylar end are smaller.

Wall formation, if it occurs, is usually centripetal, i.e., it begins at the periphery of the embryo sac and cell plates gradually extend inwards.

Cocos nucifera is the classical example of nuclear endosperm. The watery or milky liquid endosperm, which fills the large embryo-sac, contains numerous free nuclei. It is known as *liquid syncytium*. Besides free nuclei, syncytium also contains groups of multinucleate cells. These cells and the free nuclei gradually accumulate along the periphery towards the centre. The tissue thus formed is known as *coconut meat*.

The development of endosperm in *Areca catechu* is also more or less similar to that of coconut. The endosperm occupies the entire cavity of the embryo sac and it is very hard.

Endosperm haustoria develops in the chalazal coenocytic part of the embryo sac.

Cellular Endosperm

In the formation of cellular endosperm, there is no free nuclear phase as the wall formation begins right with the first division of primary endosperm nucleus. The primary endosperm nucleus may lie at the centre of the embryo sac or at the chalazal region as in *Scrophulada* and *Alectra*.

In general, the cellular endosperm is mostly confined to the dicotyledonous families. It has been proposed that where the embryo grows slowly and the seed contains an immature undifferentiated embryo, the endosperm is cellular.

The wall laid down after the first division of the primary endosperm nucleus is usually transverse, dividing the embryo sac into a micropylar and a chalazal chamber. But occasionally the wall laid down after the first nuclear division may be oblique or vertical.

The cellular endosperm is classified into the following five sub-types on the basis of the orientation of the wall laid after the first and one or two subsequent divisions of the primary endosperm nucleus.

1. The orientation of the wall formed after the first nuclear division is vertical, i.e., longitudinal to embryo sac. The second wall is also vertical but at right angles to the first. The subsequent wall formation is restricted to the micropylar end only. Thus this is an intermediate type between the nuclear and cellular endosperm and is found in *Adoxa*, *Cetranthus*, and some members of *Dipsacaceae*.

2. The orientation of the first wall is transverse and one or both the daughter cells thus formed divide vertically. This type occurs in *Scutellaria* and *Verbascum*.
3. The first division is transverse and one or both the daughter cells divide transversely. Thus in this type of cellular endosperm the first 2—3 divisions are transverse. This type of endosperm is found in *Ericaceae* and *Annonaceae*.
4. The orientation of the first wall is oblique and the two cells thus formed may be of equal or unequal size. This type is reported in *Myosotis arvensis*.
5. The orientation of the first wall is indefinite. This type of endosperm occurs in *Senecio*, *Gunnera*, etc.

A characteristic feature of the cellular endosperm is that one or more cells become specialized to function as haustoria. The haustoria are formed at the micropylar or chalazal or at both the ends and penetrate the nucellar tissue to absorb nutrition.

Helobial Endosperm

It is confined mostly to monocot families. In this type of endosperm development, after triple fusion the primary endosperm nucleus migrates to the chalazal end of the embryo sac. The wall formation after the first division of the primary endosperm nucleus results into a large micropylar and a small chalazal endosperm chamber. Further nuclear divisions are confined to the micropylar chamber, while the nucleus of the chalazal chamber remains undivided or may undergo only a few divisions. In micropylar chamber regular wall formation takes place and it becomes multicellular. But there is no wall formation in the chalazal chamber and at maturity it contains one or more disorganized nuclei. Ultimately the chalazal chamber gets crushed by the growth of the micropylar chamber.

In the helobial endosperm the haustoria usually develop from the micropylar tissue. They are tubular, unicellular but extensive outgrowths which penetrate the nucellus at the chalazal end.

Importance of endosperm

Importance for the plants

1. The endosperm is the main nutritive tissue of the seed providing nourishment to the growing embryo.
2. It can also be a source of growth factors like auxins and cytokinins to stimulate embryonic growth, as in *Zea mays*.
3. It provides protection to the growing embryo by forming a surrounding structure.

4. Due to its higher ploidy and occasional polyteny, it can synthesize larger amounts of nutritive matter.
5. It also acts as a channel for nutrient flow to the embryo by obtaining food from perisperm or normal nucleellus through haustoria.
6. It poses no competition for the growing embryo, as endosperm is a short lived tissue. However, *Brachiaria setigera*, an apomict species, is the only example where endosperm produces triploid embryos and seedlings (Muniyamma, 1978).

Importance from human perspective

1. Endosperm is the most important plant tissue for humans. Nearly 70% of dietary calories are provided to humans by endospermous matters of cereal grains, pulses and oil seeds. Thus, endosperm accounts for the economic importance of cereal grains, pulses and oilseeds.
2. Industrial oils like palm oil, castor oil, *Jatropha* oil etc. are derived from endosperm.
3. Many cereal products like corn-flakes, popcorn, beer etc. are processed from endosperm.

In the coconut and maize, the liquid endosperm contains important growth substances. It is due to the presence of these factors that coconut milk is used as a nutrient medium in culture experiments.

9. Apomixis

Introduction to apomixis

In 1903, Ostenfeld and Raunkiaer came across male sterile biotypes of *Taraxacum* and *Hieracium*, which could form seeds without pollination and fertilization.

Winkler in 1908, coined the term **Apomixis** for the phenomenon of seed production by angiosperms without fertilization.

Currently, there are two major ways, in which the term Apomixis is interpreted. Most of the plant breeders follow Winkler's definition, that is seed production without sexual process. On the other hand, the Botanists treat any asexual method of plant reproduction as Apomixis that replaces the normal sexual process partly or totally. A plant showing Apomixis is called an **Apomict**.

Types of Apomixis

Following the definition accepted by the Botanists, there are two major types of Apomixis:

1. Vegetative Reproduction
2. Agamospermy

Vegetative Reproduction

It is the use of some specialized vegetative part or propagule by the plant for proliferation. Vegetative reproduction is rather common in several angiosperms but they all are not regarded as Apomicts. To be called an apomict, an angiosperm must show partial or complete replacement of the sexual process by the asexual method. Common examples include: *Agave sp.*, *Lilium sp.* and *Fritillaria sp.*

Based on location of the propagules and essentiality of vegetative method, vegetative reproduction was sub divided into three types by Gustafsson in 1946.

1. Propagules are formed outside the floral region. The flower is normal but there is no fertilization or any seed formation. Examples: *Agave americana* and *Elodea canadensis*.

2. Propagules are formed outside the floral region. The plant is sterile. Hence, there is no fertilization or any seed formation. Examples: *Fritillaria imperialis* and *Lilium bulbiferum*.
3. Propagules are formed in the floral region, generally on the floral branches. It is also called Vegetative Vivipary. It is seen in Grasses (such as *Festuca*, *Deschampsia*) and *Allium*

Agamospermy

Here the seeds, hence the embryos, are formed but there is no sexual process preceding that. It is thus Apomixis in true Winklerian terms.

A recent study by G. Mandelkov et. al. in 2003 establishes that Agamospermy is found in at least 400 Angiosperm genera, spread over 52 families. It is most common in the polyploid genera of the following four families.

1. Asteraceae
2. Poaceae
3. Rosaceae
4. Rutaceae

Based on the source of embryo, agamospermy can be of the following types.

1. Diplospory
2. Apospory
3. Adventive embryony

Diplospory

Diplospory was described as Generative Apospory by Dr. Maheshwari in 1950. In this, the MMC does not undergo sporogenic meiosis, giving rise to diploid spore cell, that in turn divides into a diploid embryosac with a diploid egg cell. The diploid egg cell is parthenogenetic and forms a diploid embryo.

The process is as follows:

MMC → No meiosis → A Diploid Spore → Diploid embryo sac → Diploid Egg → By Parthenogenesis diploid Embryo

There are 7 types of Diplospory on the basis of meiotic abnormality.

1. Taraxacum type: Failure of meiotic synapsis, requires formation of restitution nucleus. Found in *Taraxacum*, *Erigeron* and *Arabis holbellis*.
2. Ixeris type: Failure of meiosis due to triploidy. Found in *Ixeris dentata*.

3. *Antenneria* type: MMC does not enter meiosis at all. This the most widely distributed type of diplospory. Apart from *Antenneria*, also seen in *Tripsacum* sp. and *Eupatorium* sp.
4. *Allium* type: There is a pre-meiotic chromosomal doubling, due to which the post meiotic nucleus remains diploid.
5. *Blumea* type: MMC undergoes mitotic division to form a dyad of $2n$ megaspores. The chalazal megaspore gives rise to the mature, 8-nucleate embryo sac.
6. *Elymus* type: In *Elymus rectisetus*, the megasporocyte nucleus enlarges and becomes deformed. The first pro-phase follows the nuclear deformation leading to a mitotic division resulting into a dyad of completely separated cells. The chalazal member of the dyad undergoes three rounds of mitosis to form an 8-nucleate embryo sac.
7. *Eragrostis* type: In this type, there are no meiotic stages and MMC undergoes only two rounds of mitotic division, leading to a 4-nucleate embryo sac with an egg, two synergids and one polar nucleus

Apospory

Apospory is the most common cause of apomixis in angiosperms. Dr. Maheshwari in 1950, described this phenomenon as Somatic Apospory.

In apospory, unreduced embryo sacs develop from nucellar cells (or rarely from integumentary cells) in the ovule. Several cells of the nucellus may start aposporous development but usually only one of them gives rise to a mature embryo sac. Apospory is initiated after MMC differentiation. The megaspore degenerates and the aposporous embryo sac occupies the position near the micropylar end of the ovule. The embryo develops parthenogenetically from the unreduced egg, but pollination and fertilization are required for the development of endosperm. Apospory is of common occurrence in a large number of apomicts of the grass family (*Pennisetum*, *Cenchrus*, *Poa*) and it is of two types.

The basic process of Apospory is:

A somatic cell of the ovule, such as a Nucellar or Integumentary Cell → Metamorphosis → Behaviour like a Diploid Spore → Diploid Embryo sac → Diploid Egg → By Parthenogenesis a diploid Embryo

Often aposporously produced embryo can coexist with normal sexual embryo, leading to Polyembryony.

There are two types of Apospory.

1. *Hieracium* type: The MMC behaves normally, giving rise to a normal, haploid embryo sac. At the same time, a nucellar cell also gives rise to a diploid embryo

sac - which is aposporous and 8 nucleated. Later the normal, haploid embryo sac degenerates and only the aposporous embryo sac survives.

2. **Panicum type:** It is 4 nucleated, without any antipodal apparatus. These nuclei organize into the female gametophyte consisting of a three-celled egg apparatus and a single polar nucleus; the antipodal cells are absent.

Adventive embryony

Adventive embryony is an abnormal development in which the embryo directly develops from some somatic cell of the ovule. There is never a spore like cell or an embryo sac. So, the basic process is:

A somatic cell of the ovule, such as a Nucellar or Integumentary Cell → By repeated but ordered division → Diploid Embryo

In his classification of Apomixis, Dr. Maheshwari also used the same term for this phenomenon. It is seen in Citrus and several members of Buxaceae, Cactaceae, Euphorbiaceae, Myrtaceae, and Orchidaceae - where it is the root cause of Polyembryony.

Non-Recurrent Type of Apomixis

Dr. Maheshwari also explained the Non-Recurrent Type of Apomixis, which is basically Haploid Apomixis but the offsprings produced from such seeds are sterile. Such haploid apomicts arise from parthenogenetic development from egg cell or by abnormal divisions in some other cell of the embryo sac (apogamy).

Mechanism of apomixis

During apomictic reproduction, three major developmental components are observed.

1. Generation of a cell capable of forming an embryo sac without prior meiosis (apomeiosis)
2. Fertilization-independent development of the embryo (parthenogenesis)
3. Autonomous development of the endosperm or an endosperm derived from fertilization.

The gametophytic apomicts are mostly polyploids. It has also been observed that apomixis affects only the female reproductive pathway and most apomicts are facultative, as some progeny still result from sexual reproduction.

It can be concluded that apomixis does not replace sexuality and it coexists with sexual development within the same plant. Therefore, apomixis can be viewed as a result of a relaxation of temporal and spatial constraints on sexual developmental processes.

Potential value of apomixis in agriculture

Apomixis is an attractive trait for the enhancement of crop species because it can give rise to large genetically uniform populations and perpetuates hybrid vigour through successive seed generations. Many agronomic advantages of apomixis can be foreseen:

1. the rapid generation and multiplication of superior forms through seed
2. the reduction in cost and time of breeding
3. the avoidance of complications associated with sexual reproduction, such as pollinators and cross-compatibility
4. the avoidance of complications and costs associated with vegetative propagation including the avoidance of viral transfer in plants that are typically propagated vegetatively.

However, an important risk of apomixis is that apomicts can become invasive when they are introduced in some ecological niches. Generally, apomicts have potential to be good colonizers and there are some examples of apomictic weeds and their invasiveness.

10. Patterns of embryo development

Introduction to Embryogeny

The fertilized egg is called zygote. Following a predetermined mode of development (**embryogeny**) it gives rise to an embryo, which has the potentiality to form a complete plant.

The process of embryogenesis may occur naturally in the plant as a result of sexual fertilization or asexual processes, these embryos are called **zygotic embryos** and develop into seeds, which germinate giving rise to seedlings. Plant cells can also be forced to form embryos in plant tissue culture; these embryos are called **somatic embryos**. Zygotic and somatic embryos share a number of characteristic developmental stages, however the very early steps in their development are not well correlated.

The zygotic embryogenesis is different in many ways between the Dicots and the Monocots. The variations in the developmental pattern of embryo during early embryogeny are common to monocotyledons and dicotyledons. Differences appear when the initials of plumule and cotyledon (s) are laid down.

The Process of Embryogeny in the Dicots

Preparation for the Zygotic Division (Also applicable to the monocots)

1. Soon after syngamy the larger vacuole in the zygote starts shrinking. As a result, the cell size is also reduced. This reduction may be minor as in *Capsella*, or highly significant as in cotton.
2. The decrease in cell size causes additional accumulation of cytoplasm at its chalazal end where first division of the zygote will take place.
3. The number of active dictyosomes increases for wall synthesis around the zygote. The wall, which was restricted to the micropylar end of the egg before fertilization, is now complete around the zygote.

4. The ribosomes aggregate to form polysomes, indicating the beginning of metabolic activities.
5. The zygote is now ready to divide. It is now a highly polarized, isolated cell. Its plasmodesmatal connections with the surrounding cells are blocked. The nucleus, surrounded by a large number of plastids and mitochondria, is located at the chalazal region of the cell. The micropylar end of the zygote (basal pole) is occupied by one or more vacuoles, and the number of cell organelles in this region is extremely small.

Zygotic Division

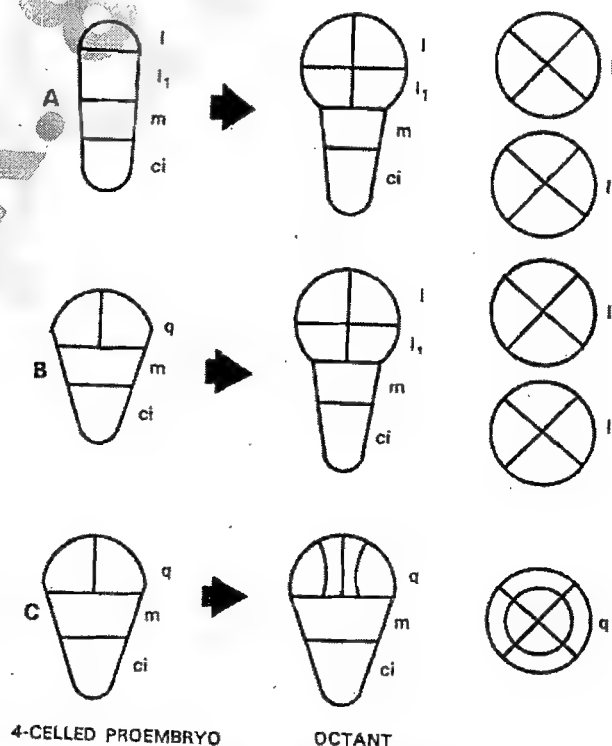
Plant embryogenesis begins with an **asymmetric cell division**, resulting in a smaller **apical** (terminal) cell and a larger **basal** cell. This first asymmetric division provides polarity to the embryo. Most of the plant embryo develops from the apical (terminal) cell. The **suspensor** develops from the basal cell. The suspensor anchors the embryo to the endosperm and serves as a nutrient conduit for the developing embryo.

In the majority of angiosperms the zygote divides transversely, resulting in a small apical cell (conventionally designated **ca**) toward the interior of the embryo sac and a large basal cell (conventionally designated **cb**) toward the micropyle. Rarely, the division of the zygote may be vertical (Loranthaceae) or oblique (*Triticum* sp).

From the 2-celled stage until the initiation of organs the embryo is commonly called **proembryo**.

Proembryo

In a 2-celled proembryo, the basal cell (**cb**) either remains undivided, or it undergoes a transverse division to form two cells **m** and **ci**. In the latter case, depending on whether the division of the apical cell (**ca**) is transverse or vertical, the 4-celled proembryo is linear or T-shaped, respectively. In the linear proembryo the two daughter cells of **ca** (**l**, **l₁**), by two vertical divisions at right angles to each other, give rise to an octant with two superposed tiers (**l**, **l₁**) of four cells each. An octant of similar



4-CELLED PROEMBRYO OCTANT
FIGURE 1: The various ways of octant formation

configuration is formed by the T-shaped proembryo by one transverse division and one vertical division. The T-shaped proembryo can also form an octant of a different configuration, in which all the eight cells are included in the same tier (q); an axial quadrant is surrounded by four peripheral cells. Thus, in angiosperms two types of octant configurations occur; (a) the component cells are arranged in two superposed tiers of 4-cells each (*Beta*, *Capsella*, *Poa*, *Sagittaria*), for (b) all the 8-cells occur in a single tier (*Lactuca*, *Muscari*). As is evident from the cited examples, both types of octants occur in monocotyledons as well as in dicotyledons. It is at the octant stage of the proembryo that the destinies of various cells become determined.

Types of Embryogeny in Dicotyledons

Based on the plane of division of the apical cell in the 2-celled proembryo, and the contribution of the basal cell (cb) and the apical cell (ca) in the formation of embryo proper, five chief types of embryogeny have been recognized by Maheshwari (1950). A new type called Piperad type has been added by Johansen in 1951. This classification also applies to several monocot plants as well. The classification is outlined below.

Group A: The apical cell of the 2-celled proembryo divides longitudinally.

Type 1: The basal cell plays only a minor role or none in the subsequent development of the embryo proper—Crucifer Type or Onagrad Type (e.g., Annonaceae, Brassicaceae, Onagraceae, Pedaliaceae, Ranunculaceae, Scrophulariaceae).

Type 2: The basal cell and apical cell both contribute to the development of embryo—Asterad Type (e.g., Asteraceae, Balsaminaceae, Violaceae, Vitaceae).

Group B. The apical cell of the 2-celled proembryo divides transversely.

Type 1: The basal cell plays only a minor role or none in the subsequent development of the embryo proper. The basal cell usually forms a suspensor—Solanad Type (e.g., Campanulaceae, Linaceae, Solanaceae, Theaceae).

Type 2: The basal cell plays only a minor role or none in the subsequent development of the embryo proper. The basal cell undergoes no further division, and the suspensor, if present, is always derived from the apical cell—Caryophyllad Type (e.g., Caryophyllaceae, Crassulaceae, Haloragaceae).

Type 3: The basal and apical cells both contribute to the development of embryo—Chenopodiad Type (e.g., Boraginaceae, Chenopodiaceae).

These five types of embryogeny refer to those plants where first division of the zygote is transverse, so that an apical cell and a basal cell are formed. Johansen (1950) has recognized a sixth type of embryogeny, called Piperad Type, which includes those cases where first division of the zygote is vertical (Loranthaceae, Piperaceae).

Often the type of embryogeny is constant throughout a family. Rarely, however, the same species may show more than one well established trend well as Crucifer Type of embryogeny occur regularly.

Embryogenesis in dicots

The major stages of dicot angiosperm embryogeny are shown below in a simplified manner below in Figure 2.

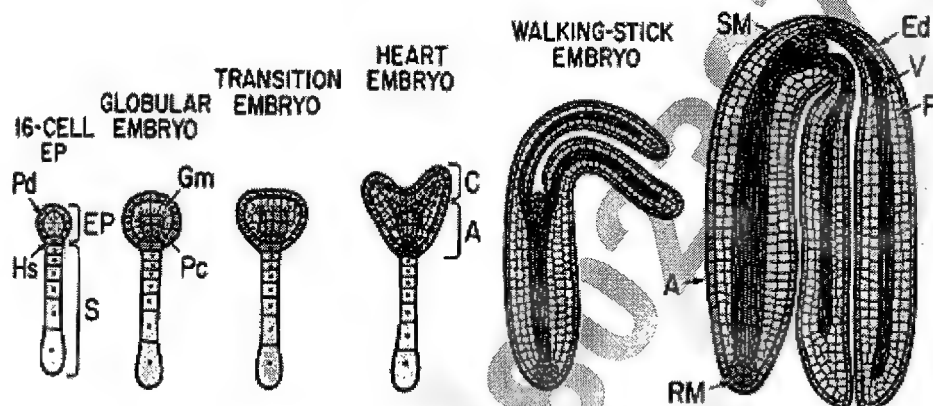


FIGURE 5: THE MAJOR STAGES OF DICOT ANGIOSPERM EMBRYOGENY

Case Study of Dicot Embryogeny

To illustrate complete development of a dicotyledonous embryo the work of Bhandari and Asnani (1968) on *Ceratocephalus falcatus* (Ranunculaceae) is described below. In this species the embryogeny is of the Onagrad Type.

1. The zygote divides transversely, forming a small apical cell (ca) and a large basal cell (cb).
2. (cb) divides transversally to give rise to two cells called ci and m.
3. Cell ca undergoes a vertical division giving rise to two juxtaposed cells. Thus, a T-shaped, 4-celled proembryo is formed.
4. Of the two daughter cells of cb, cell ci divides transversely giving rise to n and n'. These two cells divide further forming a linear row of 3 or 4-celled suspensor.
5. Cell m and its derivatives divide by a vertical division to form 4-6 cells.
6. Oblique periclinal divisions in each of these cells result in an inner set of cells (the initials of root apex) and an outer set of cells (the initials of root cap).
7. In the meantime the daughter cells of the apical cell divide by another vertical division at right angles to the first division, forming a quadrant q.
8. A transverse division of the quadrant results in an octant arranged in two tiers (l, l_1) of four cells each.
9. Vertical divisions in tiers l and l_1 give rise to a globular proembryo.

10. Periclinal divisions in the peripheral cells of the globular proembryo demarcate a single-layered dermatogen, the future epidermis.
11. Cells of the tier l differentiate the initials of plumular zone. As a result, in the mature embryo the plumule is enclosed at the base of the two cotyledons.
12. The tier l_1 finally forms the hypocotyls – radical axis.

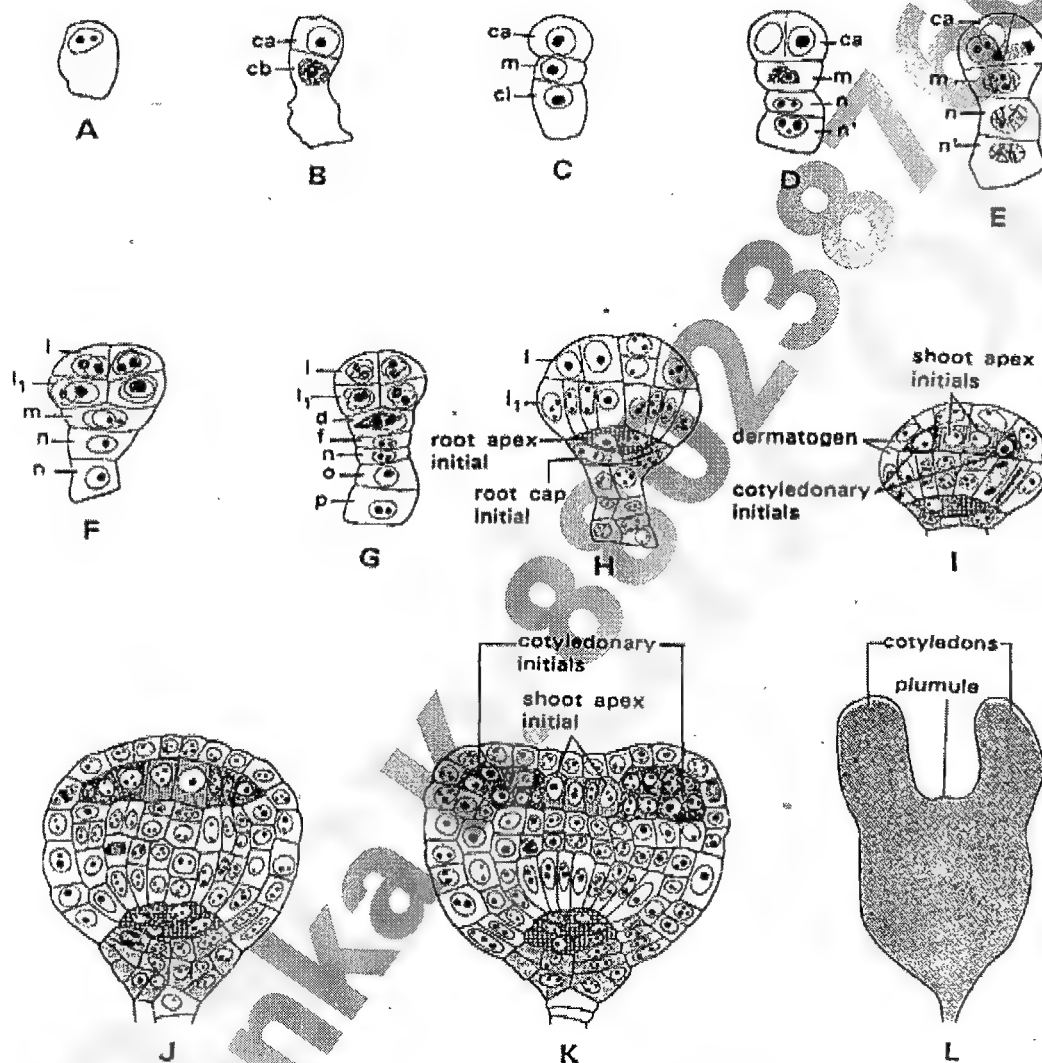


FIGURE 6: EMBRYOGENY IN *CERATOCEPHALUS FALCATUS*

Embryogeny in Monocotyledons

As mentioned earlier, the development of embryo up to the octant stage is almost similar in monocotyledons and dicotyledons. The differences appear later.

5. Two vertical divisions at right angles to each other in the two distal cells (c, m) lead to the formation of two superposed tiers (q, m) of four cells each.
6. In the meantime cell ci divides transversely to give rise to n and n'.
7. Whereas cell n divides vertically, n' undergoes transverse division giving rise to two cells, o and p.
8. The latter (p) undergoes another transverse division producing cells h and s.
9. The quadrant q divides by a periclinal division cutting a four-celled dermatogen surrounding the four axial cells.
10. The cells in the tier m divide by vertical and transverse division and become two tiered. At this stage the proembryo is slightly spherical.
11. Now onwards it elongates appreciably due to mainly transverse divisions in the tiers m and n. At the stage when embryo becomes oval the central core of cells in the tiers q, m, and n differentiates into plerome initials.
12. At the eight celled stage of the tier q (4 axial cells and 4 circumaxial cells) three of the axial cells divide faster than the fourth one. This disturbs the symmetry of the proembryo, and its top becomes notched. The rapidly growing portion of the tier q forms the single cotyledon. And the slow growing tissue, derived from the fourth axial cell, gives rise to the initials of epicotyl.
13. The radicle is organized from the derivatives of n.

Methods to study plant embryogenesis

The angiosperm zygote is embedded within the ovule and ovary and thus is not readily accessible for experimental manipulation. The following approaches, however, can yield information on the formation of the plant embryo:

1. **Histological studies** of embryos at different stages show how carefully regulated cell division results in the construction of an organism, even without the ability to move cells and tissues to shape the embryo.
2. **Culture experiments** using embryos isolated from ovules and embryos developing de novo from cultured sporophytic tissue provide information on the interactions between the embryo and surrounding sporophytic and endosperm tissue.
3. **In vitro fertilization experiments** provide information on gamete interactions.

4. **Biochemical analyses** of embryos at different stages of development provides information on such things as the stage-specific gene products necessary for patterning and establishing food reserves.
5. **Genetic and molecular analyses of developmental mutants** characterized using the above approaches have greatly enhanced our understanding of embryonic development.
6. **Clonal analysis** involves marking individual cells and following their fate in development. For example, seeds heterozygous for a pigmentation gene may be irradiated so that a certain cell loses the ability to produce pigment. Its descendants will form a colorless sector that can be identified and related to the overall body pattern.

Genetic Control

All the aspects of genetic control of angiosperm embryogeny are still not clear, but the following facts are well established. The study of embryo mutants in maize and *Arabidopsis* has been particularly helpful.

1. Investigations of suspensor mutants (*sus1*, *sus2*, and *raspberry1*) of *Arabidopsis* have provided genetic evidence that the suspensor has the capacity to develop embryo-like structures. A signal from the embryo proper to the suspensor may be important in maintaining suspensor identity and blocking the development of the suspensor as an embryo.
2. Maternal effect genes play a key role in establishing embryonic pattern plant embryogenesis also. Yet, the question is not clearly answered due to at least three potential sources of influence: sporophytic tissue, gametophytic tissue, and the polyploid endosperm. All of these tissues are in close association with the egg/zygote.
3. Sporophytic and gametophytic maternal effect genes have been identified in *Arabidopsis*, and it is probable that the endosperm genome influences the zygote as well. The first maternal effect gene identified, *SHORT INTEGUMENTS 1* (*SIN1*), must be expressed in the sporophyte for normal embryonic development (Ray et al. 1996).
4. Two transcription factors (FBP7 and FBP11) are needed in the *Petunia* sporophyte for normal endosperm development.
5. A female gametophytic maternal effect gene, *MEDEA* (from Polycomb gene group), is also involved whose products alter chromatin, directly or indirectly, and affect transcription.

6. The *keule* gene of *Arabidopsis* is necessary for the dermal system development.
7. Genetic evidence indicates that the formation of the shoot and root meristems is regulated independently.
8. The *dek23* maize gene and the *shootmeristemless (STM)* gene of *Arabidopsis* are necessary to initiate a shoot meristem.
9. The *STM* gene, which has been cloned, is expressed in the late globular stage, before cotyledons form.
10. *HOBBIT* gene in *Arabidopsis* (Willemsen et al. 1998) affect the hypophysis derivatives and and initiates root meristem function.

11. Polyembryony

What is Polyembryony?

In general biology, Polyembryony is a condition in which two or more embryos develop from a single fertilized egg.

However, with respect to seed plants, Polyembryony means the presence of more than one embryo in a single seed. Any embryo more than one is called a supernumerary embryo. In plants, such an embryo may arise from sources other than a fertilized egg too.

The additional embryos within a polyembryonic seed do not always mature. They may become arrested at very early stages or may degenerate during the course of seed development.

The first case of polyembryony was reported in certain orange seeds by Antoni van Leeuwenhek (1719).

Occurrence

Polyembryony is far more common in gymnosperms in comparison to angiosperms. Except for a few taxa (*Citrus*, *Mangifera*), polyembryony occurs only as an abnormal feature among the angiosperms.

Types

Braun (1859) gave a survey of 58 cases of polyembryony recorded in the botanical literature at that time and referred them to four categories on the basis of the origin of the additional embryos. Polyembryony in angiosperms may arise by:

1. Cleavage of proembryo
2. Formation of embryos by cells of the embryo sac other than the egg
3. Development of more than one embryo sac within the same ovule
4. Activation of some sporophytic cells of the ovule.

Cleavage of the proembryo

The equational cleavage proembryo leads to the establishment of supernumerary embryos. Cleavage polyembryony is widespread among gymnosperms. In angiosperms this feature is less frequent.

Among angiosperms cleavage polyembryony is quite common in orchids. In *Eulophia epidendreae*, Swamy (1943) recorded three different modes of supernumerary embryo formation:

1. The zygote divides irregularly to form a mass of cells of which those lying toward the chalazal end grow simultaneously and give rise to many embryos.
2. The proembryo forms small buds or outgrowths which may themselves function as embryos.
3. The filamentous embryo becomes branched, and each branch forms an embryo.

The formation of plural embryos during seed germination is known in *Vanda*. Suspensor Polyembryony is seen in *Exocarpus*, a member of Santalaceae.

Embryos from cells of the embryo sac other than the egg

In this category the most common source of additional embryo is the synergid. Depending on whether it arises from fertilized synergid or unfertilized synergid, the embryo may be diploid or haploid.

In *Arisolochia bracteata*, *Poa alpina* and *Sagittaria graminea* besides the egg and the polars one or both the synergids may get fertilized and form diploid supernumerary embryos.

Embryos arising from unfertilized synergids are known in *Argemone mexicana* and *Phaseolus vulgaris*.

Formation of embryos from antipodal is also known but rather rare. It has been observed in *Paspalum scrobiculatum*, *Ulmus americana* and *U. glabra*.

Most of the report concerning endosperm cells forming embryos are doubtful. However, *Brachiaria setigera*, an apomict species, is the only example where endosperm produces triploid embryos and seedlings (Muniyamma, 1978).

Development of more than one embryo sac within the same ovule

Multiple embryo sacs in an ovule may arise from:

1. Derivatives of the same megaspore mother cell,
2. Derivatives of two or more megaspore mother cells, or
3. Nucellar cells.

Formation of twin embryo sacs within an ovule is known in *Casuarina equisetifolia*, *Citrus* and *Poa pratensis* and *Pennisetum cilliare*.

The members of the family Loranthaceae lack a conventional ovule. Numerous embryo sacs develop concurrently in the same ovary and their tips, carrying the egg apparatus, grow up to various heights in the style. After fertilization, the Polyembryony results in these plants.

Activation of some sporophytic cells of the ovule

The embryos arising from the maternal sporophytic tissues (outside the embryo sac) are called adventive embryos. The only maternal tissues which are known to form adventive embryos are the nucellus and the integuments.

Besides the more popular examples of *Citrus* and *Mangifera*, nucellar polyembryony occurs in *Opuntia dillenii* and *Trillium undulatum*. Some species of *Citrus* are monoembryonate (*C. grandis*, *C. limon*) while others are polyembryonate (*C. microcarpa*, *C. reticulata*). Seeds with as many as 40 embryos have been recorded in *C. unshiu*.

Adventive embryogenesis in *Citrus* can be divided into four steps (1) formation of Adventive Embryo Initial Cells (AEICs), (2) differentiation of AEICs to acquire the appearance of a zygote, (3) division of AEICs, and (4) development of adventive embryos.

Causes of polyembryony

Many theories seek to explain the occurrence of polyembryony but none is sufficiently validated. In different theories polyembryony has been ascribed to:

1. Hybridization
2. Necrohormones
3. Effect of recessive genes, etc.

Haberlandt (1921, 1922) proposed the “necrohormone theory”. He regarded the degenerating cells of the nucellus as source of stimulus for attempted to induce adventive polyembryony in *Oenothera*.

Leroy (1947) thought that polyembryony in mango was caused by one or more recessive genes.

According to Frusato *et al.* (1957), the embryo number in *Citrus* seeds may be influenced by the following factors:

1. Age of the tree; increasing in older trees.
2. Fruit-set; being higher in years of higher fruit-set.

3. Nutritional status of the plant; Polyembryony decreasing with reduced food supply.
4. Orientation of the branch of the tree; being higher on northern than on southern branches.

The monoembryonate condition in some species of *Citrus* has been ascribed to the synthesis and release of certain volatile and non-volatile embryogenic inhibitors in their ovules which do not occur in the ovules of polyembryonate species. The non-volatile component of the inhibitors has been identified with IAA, ABA, and GA₃.

Applications

1. Adventive embryony provides uniform seedlings of the parental types, as obtained through vegetative methods.
2. Somatic embryos can be used to produce artificial seeds.
3. Adventive embryos serve to preserve a particular type of genotype.

The above applications have immense value to horticulture.

12. Pollen storage

Introduction

Pollen grains can be stored for extended periods under suitable conditions to maintain their viability and used when required. Pollen storage methods thus seek to achieve the longest viability of pollens and the possibility of their use as, when and where required.

There is no single storage method that can be applied to all the pollen types. Different species respond well to different storage methods.

The methods of pollen storage

The important methods used are briefly summarized below.

Storage under low temperature (+4 to -20°C) and low humidity (<10% RH)

- (1) Pollen grains are stored in suitable airtight vials containing an appropriate dehydrating agent such as dry silica to maintain low RH (less than 10%).
- (2) The sealed desiccators are then kept in a refrigerator or deep-freeze.

Benefits

This method is very convenient and effective for short-term storage (for a few weeks/months). It has been used extensively by amateur horticulturists.

Storage under subfreezing temperatures (-20°C) is effective for storing pollen of several species for more than a year – such as *Citrus* spp., *Corylus* spp., *Juglans* spp. etc.

Limitations

Pollen grains of cereals in general cannot withstand desiccation. They need to be stored under high RH in the refrigerator.

Storage of freeze-dried / vacuum-dried pollen

- (1) Freeze-drying involves rapid freezing of pollen (-60 to -80°C) and gradual removal of water under sublimation. In this method frozen water is allowed to sublime under reduced pressure or vacuum.
- (2) The dried pollen is usually stored at subzero temperatures.

Benefits

This method has been effective for long-term storage of pollen grains of a number of species (*Allium cepa*, *Amaryllis* sp., *Antirrhinum majus*, *Beta vulgaris* etc.) except those of cereals.

Storage under ultralow temperature / cryopreservation

In this method,

- (1) Pollen grains are dried to bring their water content below a threshold level, and
- (2) The stored in liquid nitrogen (-196° C).

Benefits

This has been the most effective method for longterm storage for a large number of species, including:

Rhododendron, *Beta vulgaris*, *Brassica oleracea*, *Capsicum annum*, *Carya illinoensis*, *Carica papaya*, *Helianthus annus*, *Humulus lupulus*, *Pistacia* sp., *Prunus persica* etc.

Limitations

Initial attempts to cryopreserve pollen grains of cereals were not successful largely because of their susceptibility to desiccation. However, by using pollen dryer in which air of 20°C and 20-40% humidity is blown through pollen, Barnabas and Rajki (1981) were able to successfully store pollen of many cereals for over 10 years.

Storage in organic solvents

This is a very simple method of pollen storage, first reported by Iwanami (1972) and Iwanami and Nakamura (1972).

- (1) Pollen grains are dried over silica; and
- (2) Then stored in organic solvents (taken in airtight vials) and maintained in a refrigerator or deep-freeze. At this step, non-polar solvents such as hexane, cyclohexane and diethyl ether are more effective.
- (3) After the storage period, pollen grains are recovered by filtration or evaporation of the solvent and used for pollination or for conducting any other viability test.

Applications

It has successfully been applied on several cereal species like *Zea mays*, *Triticum spp.* etc.

Limitations

The number of species in which this method has been effective is thus far limited.

Applications of pollen storage

Pollen storage has important applications in both basic and applied areas of reproductive biology. These are some of them:

- (1) Overcoming crossability barriers imposed due to temporal isolation of the parent species.
- (2) Overcoming crossability barriers imposed due to spatial isolation of the parent species.
- (3) Eliminating the need to grow pollen parents continuously in breeding programmes.
- (4) Implementing supplementary pollinations to sustain yield.
- (5) Preserving genetic resources and providing germplasm for international exchange.
- (6) Ensuring availability of pollen throughout the year for studies on various aspects of pollen biology and pollen allergy.

The need to establish pollen banks has long been emphasized. They would ensure availability of pollen of the desired species at any time of the year and at any place. Pollen banks would greatly facilitate the breeding programme, particularly of tree species.

13. Applications of palynology

What is Palynology?

There is no clear consensus on a definition of the term palynology.

The term **Palynology** was first used by **Hyde and Williams** in 1944, they defined it as "the study of pollen and other spores and their dispersal, and applications thereof."

However, in the recent international literature, the term has been defined more broadly as the "study of organic microfossils – also called **Palynomorphs**" (Alfred Traverse, 1989).

Most of the Indian embryologists including Dr. Maheshwari, Dr. Johari, Dr. Sivanna and Dr. Bhojwani have treated this term in the classical sense of Pollen Analysis and Study only.

At the same time, most of the western authors are in consensus that palynology is the branch of science dealing with microscopic, decay-resistant remains (called Palynomorphs) of certain plants and animals. Such palynomorphs include pollens, spores of fungi, algae, bryophytes, fossils of diatoms etc.

Palynological Methods

Palynomorphs are extracted from rocks and sediments both physically and chemically. The physical method is **wet sieving**, and the chemical method includes **digestion** to remove the non-organic fraction.

Samples are then cleaned and mounted on microscope slides. They are examined using light microscopy or scanning electron microscopy. Once the palynomorph has been identified it can be plotted on a diagram which is then used for interpretation.

Palynology uses many techniques from other related fields such as geology, botany, paleontology, archaeology, pedology, and geography.

Applications

There are a several •botanical, •geological, •commercial and •medicine & forensic fields in which palynology can be applied.

Botanical applications

1. Determining the vegetational history of an area

2. Taxonomy
3. Evolution
4. Horticulture and Breeding

Determining the vegetational history of an area: The exine of a pollen grain is very characteristic for a family, genus or sometimes even species. It is also very resistant to decay. Thus, the pollens falling on rapidly accumulating sediment, anaerobic water or peat are preserved. These depositions generally give a very good picture of the surrounding regional vegetation at an older period.

Taxonomy: Sculpturing patterns of the exine form a very consistent taxonomic feature – which can be used in plant identification with accuracy. A number of taxonomic problems have been solved using palynological data.

Based on pollen morphology, angiosperm families are characterized as **Eurypalynous** (if the family is showing different pollen characteristics within itself, such as Asteraceae) or **Stenopalynous** (if the family shows a consistency of pollen features among its members, such as Brassicaceae).

Based on pollen morphology, the family Butomaceae has been separated from the family Alismaceae. Similarly, the placement of *Trapa* in a separate family Trapaceae has been supported by palynological data also apart from various embryological data also.

Evolution: If two families differ from one other greatly in pollen features, then they most probably represent different evolutionary lines. In drawing evolutionary conclusions, the primary palynological features (such as aperture patterns and exine sculpturing) are most frequently considered.

Horticulture and Breeding: For mass scale breeding programmes, pollen banks are maintained. Pollination management is one of the most fundamental steps in hybridization experiments. A good pollen source is a plant that provides compatible, viable and plentiful pollen and blooms at the same time as the plant that is to be pollinated or has pollen that can be stored and used when needed to pollinate the desired flowers.

Geological applications

1. Geochronology
2. Biostratigraphy
3. Paleoecology
4. Quaternary Palynology
5. Archaeopalynology

Geochronology is dating of rocks. Palynoflora are used to date rocks because they are reliable indicators of the time ranges.

Biostratigraphy is correlation of rock sections based on palynoflora. This aspect of palynology is the most important economically, as it is the *fundamental principle behind Oil Geology*. Proper identification of indicative palynomorphs could lead to the discovery of oil, coal, and gas deposits. The colouration and type of palynomorph represents the thermal maturity and hydrocarbon potential of the area.

Paleoecology is study of past environments. Because most of the palynomorphs are sensitive to any minor fluctuation in their surroundings, they are highly indicative of the environment in which they are deposited. The advantage of palynomorphs over other fossils is their widespread distribution; they can be found in either terrestrial, freshwater, saltwater, and estuary sources of sedimentary rocks.

Quaternary palynology: Although very similar to the purposes of paleoecology, Quaternary deals more with more recent environmental and climate change.

Archaeopalynology is somewhat related to the quaternary palynology. It refers to the palynological study of **human impact on the environment**. Pollen analysis of lakes and bogs may be used to study humans as agents of vegetation change rather than causes such as climate.

Commercial applications

Melissopalynology is the study of honey and any pollen contained in it. The pollens indicates the source plant. By studying the pollen in a sample of honey, it is possible to gain evidence of the geographical location and genus of the plants that the honey bees visited. Generally, melissopalynology is used to combat fraud and inaccurate labelling of honey. Information gained from the study of a given sample of honey (and pollen) is useful when substantiating claims of a particular source for the sample. Monofloral honey derived from one particular source plant may be more valuable than honey derived from many types of plants. The price of honey also varies according to the region and plant it comes from.

Pharmacognosy is the study of medicines from natural sources. In this field, palynology is applied to gain information on the source plant. Generally, pollen data are applied by Pharmaceutical companies to combat fraud about the source plant.

Medical and Forensic applications

Allergy studies: Some pollen grains cause allergic responses in some individuals. Generally pollens that cause allergies are those of anemophilous, because the lightweight pollen grains are produced in great quantities for wind dispersal. Breathing air containing these pollen grains brings them into contact with the nasal passages.

Studies of the geographic distribution and seasonal production of pollen, can help sufferers of allergies such as hay fever.

Forensic palynology is the study of pollens and powdered minerals, their identification, and where and when they occur, to ascertain that a body or other object was in a certain place at a certain time. For instance, a dead body may be found in at one place, and the clothes may contain pollen that was released in some other place. That indicates that the body was moved.